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TSCA Chemical Assessment Series. Assessment of Testing Needs: Chlorinated Benzenes. Support Document for Proposed Health Effects Test Rule Toxic Substances Control Act. Section 4

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TSCA Chemical Assessment Series

Assessment of Testing Needs: Chlorinated Benzenes

Support Document for Proposed Health Effects Test Rule Toxic Substances Control Act Section 4

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Since chlorobenzenes are used as chemical intermediates and for other industrial purposes as well as in consumer products, there is very broad potential exposure. Thus, there is known or potential exposure of workers involved in chlorobenzene production, processing, and use, and of the general population, both directly from consumer products and indirectly through the environment. For this reason and on the basis of limited toxic effects studies, EPA has proposed that certain chlorobenzenes be tested to assess their potential to cause chronic, reproductive, teratological, and oncogenic effects. Following resolution of methodology issues the Agency has raised, EPA will propose at a later date test rules for neurotoxic and mutagenic effects. Further, the Agency has decided not to propose test rules for acute toxicity and epidemiological studies.

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SUPPORT DOCUMENT
HEALTH EFFECTS TEST RULE: CHLORINATED BENZENES

ASSESSMENT DIVISION
OFFICE OF TOXIC SUBSTANCES
Washington, D.C. 20460

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
WASHINGTON, D.C. 20460

PREFACE

Under the authority of the Toxic Substances Control Act (TSCA), the Environmental Protection Agency has proposed the requirement of health effects testing of a representative group of chlorinated benzenes (Federal Register, June 28, 1980). This action follows on the recommendations of the Interagency Testing Committee. The Agency has reviewed the available information, including TSCA Section 8(d) submissions, on chlorinated benzenes and discussed the significant scientific and economic issues both in Agency Workgroup meetings and in public meetings. The results of this effort are reflected in this document which supports the proposed health effects test rule with specifics from the literature and rationales for decisions.

The EPA encourages all interested parties to review the scientific and economic reasoning expressed in the Support Document and provide comment to the Agency. Such contributions can significantly benefit the development of the Final Health Effects Test Rule. All comments will be carefully reviewed by EPA, and all major points will be addressed in the final Support Document.

Written comments should bear the document number EPA 560/11-80-014 and should be submitted to the Document Control Officer (TS-793), Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency, 401 M St., SW, Washington, DC 20460.

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INTRODUCTION

In October 1977, the Interagency Testing Committee recommended in its first report to the EPA that monochlorobenzene and the three isomers of dichlorobenzene be given priority consideration for the development of testing requirements under Section 4 of TSCA (CEQ 1977). In its third report to EPA in October 1978, the Committee made similar testing recommendations for the trichlorobenzenes, tetrachlorobenzenes, and pentachlorobenzene (TSCA ITC 1978). This document presents detailed support for EPA's decision: (1) to propose test rules to assess the potential of chlorinated benzenes to cause chronic, reproductive, morphological teratological (birth defect), and oncogenic effects; (2) to propose test rules at a later date for neurotoxic effects, behavioral teratological effects, and metabolism studies, following resolution of methodology issues raised in the Preamble and in this document; (3) not to propose test rules for acute toxicity and epidemiological studies; (4) to have the EPA sponsor certain lower level mutagenicity tests.

It should be noted that while hexachlorobenzene is a chlorinated benzene, it was not included with the other chlorinated benzenes in the Testing Committee's recommendations. This substance has been evaluated by a separate Agency process, and referred to EPA's Office of Solid Waste for control of the major source of hexachlorobenzene release to the environment under regulations promulgated on May 19, 1980 (44 FR 33063). This document does not, therefore, consider the need for health effects testing of hexachlorobenzene, and the term "chlorinated benzenes" when used in this document does not include hexachlorobenzene as a member of the test rule category. However, information on hexachlorobenzene is included wherever it is relevant to the technical analysis.

In this document the exposure aspects section (Section II) contains separate discussions on monochlorobenzene, the dichlorobenzenes, the trichlorobenzenes, the tetrachlorobenzenes, and pentachlorobenzene. In Section III on health effects, all chlorobenzenes for which data are available are discussed together for each effect.

The EPA is aware that guidelines for the level of exposure to some chlorinated benzenes have been prescribed by the American Conference of Governmental Industrial Hygienists (ACGIH), the Food and Drug Administration, and the EPA [Clean Water Act, Safe Drinking Water Act, and Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)] and that the Department of Transportation has regulations for handling chlorobenzenes and for reporting accidental spills. These rules are based on existing data; the results of the testing proposed by EPA may necessitate additional regulations or changes in existing guidelines, standards, and regulations. A list of the existing guidelines for occupational exposure is attached as Appendix C.

Non-Confidential information from the TSCA §8(b) Chemical Inventory is cited in this document. For a discussion of the use and limitations of this information, the reader should refer to Appendix A.

SUMMARY

Exposure

The total annual production of chlorobenzenes is on the order of 450 million pounds or about half of the available capacity. Most of the production is of mono- and dichlorobenzenes; nevertheless, annual production of the commercially important tri- and tetrachlorobenzenes and pentachlorobenzene is in the millions of pounds. Total chlorinated benzene imports range up to several million pounds each year.

Chlorinated benzenes are produced by the controlled catalytic chlorination of benzene; the reaction conditions selected determine which chlorobenzenes are obtained. There are always some unwanted chlorobenzenes produced in addition to the desired product(s). Some of these by-products are recycled in order to produce higher chlorinated benzenes, while others have commercial uses. Most commercial chlorinated benzenes can thus be expected to contain some congeneric and/or isomeric chlorobenzenes as impurities. Workers involved in chlorinated benzene production and in their uses are potentially exposed to various members of the category, and various members may enter the environment as emissions and production wastes.

The potential release of chlorinated benzenes to the environment is very large because of the combination of high annual production and a wide variety of industrial and consumer uses. Many industrial uses and disposal practices may result in the ultimate discharge of chlorinated benzenes into the environment rather than in their recovery and reuse. Much of this release occurs to the atmosphere, but nearly all the chlorinated benzenes have been detected in the aquatic environment. Adsorption from water onto solids occurs to some extent. Atmospheric decomposition of some chlorinated benzenes occurs fairly readily, and there is some evidence of degradation by microorganisms under certain conditions; in most cases environmental transformation products have not been identified.

Since chlorinated benzenes are used as chemical intermediates and for other industrial purposes as well as in consumer products, there is very broad potential exposure. Thus, there is known or potential exposure of workers involved in chlorobenzene production, processing, and use, and of the general population, both directly from consumer products and indirectly through the environment.

Acute Effects

The Agency believes that the acute effects of the chlorinated benzenes, chiefly tissue irritation and nervous system depression, are adequately characterized as a result of human case studies and tests in several animal species. Further acute testing is unnecessary.

Subchronic/Chronic Effects

A number of subchronic studies were reported which indicate that the chlorinated benzenes produce damage to the liver and the hematopoietic system in humans and several animal species and to the kidney in dogs and rats. No reports of chronic studies were found. The data from these subchronic studies are inadequate to define the hazards of chronic exposure to chlorinated benzenes. Therefore, EPA is proposing that 90-day subchronic studies be done using rats, the most sensitive of species thus far tested. No chronic studies are being proposed because EPA believes that for chlorinated benzenes the vast majority of the chronic effects will appear in a 90-day study. Subchronic testing is not being proposed for pentachlorobenzene, which has been adequately tested.

Neurotoxic Effects

There are studies indicating adverse effects on the central nervous system of humans and animals after exposure to some chlorobenzenes. Since the data are not adequate to provide a complete characterization and assessment of the hazard, testing is desirable. But, because the Agency has not yet developed test standards for such testing, EPA is not proposing specific neurotoxicity or behavioral effects testing at this time.

Metabolism Studies

The metabolism studies available deal primarily with the products of chlorinated benzene metabolism and provide little information on the pharmacokinetic aspects. The studies lead EPA to the conclusion that the chlorinated benzenes are metabolized at least in part to epoxide (arene oxide) intermediates.

EPA is soliciting comments on the appropriateness of and approach to studies (a) to determine the distribution of chlorinated benzenes to tissues and organs, (b) to learn the rates of their clearance from these tissues, and (c) to ascertain whether or not chlorinated benzenes form covalent compounds with macromolecules, particularly in the brain and gonads and in organs from which excretion is slow.

Reproductive Effects

Studies in rats of hexachlorobenzene, which is structurally related to the chlorinated benzenes, have revealed that the substance passes the placenta, appears in the milk supply, and affects fertility. Further, ovarian effects have been noted in monkeys treated with hexachlorobenzene and in rats treated with monochlorobenzene. On the basis of available data and of structural relationships, EPA suspects that exposure to the chlorinated benzenes may cause reproductive effects. Because the existing data are insufficient to determine and predict effects on fertility, EPA proposes reproductive studies to develop additional data for chlorinated benzenes except 1,2,4-trichlorobenzene on which testing has recently been performed.

Teratogenic Effects: Morphological and Behavioral

Hexachlorobenzene causes teratogenic effects in mice. Pentachlorobenzene in rats causes rib abnormalities which are dose-related. Certain phenolic metabolites of the chlorinated benzenes are also known to cause embryo- and fetotoxic responses in rats. This evidence causes EPA to propose testing to evaluate the morphological teratogenic potential of chlorinated benzenes except pentachlorobenzene, which has been adequately tested.

Chlorinated benzenes are also known to be non-specific central nervous system (CNS) depressants in adults, and they or their toxic metabolites are likely to cross the placenta. Because the CNS is especially susceptible to toxic insult during its development, EPA is considering the requiring of behavioral teratogenicity testing of chlorinated benzenes. The Agency desires comments on the behavioral tests suggested in this document.

Mutagenic Effects

Evidence indicates that the chlorinated benzenes are mutagenic in certain bacterial and eukaryotic systems that detect gene mutations, produce differential cell killing in DNA repair-deficient strains of bacteria, and induce C-mitosis and chromosomal breaks in plant systems. For EPA to determine whether the chlorinated benzenes pose a genetic hazard to humans, the Agency will sponsor some initial, relatively inexpensive tests to determine whether these substances cause gene or chromosomal mutations in higher organisms. The Agency will arrange for this testing because it has not yet proposed test standards for some mutagenicity tests or decision criteria for a mutagenicity testing sequence. After evaluating the test results, EPA will decide whether to propose further testing to provide information for mutagenicity hazard assessments on chlorinated benzenes.

Oncogenic Effects

The weight of suggestive evidence leads EPA to the conclusion that chlorinated benzenes may be oncogenic. This evidence includes: (a) case reports of human leukemia, (b) positive mutagenicity test results which may have a correlation with oncogenicity, (c) structural and metabolic similarities with known oncogens, and (d) reports of tumor enhancement potential. Therefore, EPA is proposing that the chlorinated benzenes be tested for oncogenicity in a two-year study using rodents. Monochlorobenzene and *o*- and *p*-dichlorobenzene are excluded from the proposed testing because of bioassays now planned or in progress at the National Cancer Institute.

I. Chemical Identity

A. Category Definition

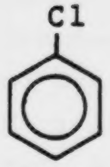
In EPA's proposed test rule for the "chlorobenzenes" and its Support Document, "chlorinated benzenes" and "chlorobenzenes" mean the group of substituted benzene compounds in which one to five hydrogen atoms of benzene are replaced by chlorine atoms, with no substituents present other than chlorine and hydrogen. As explained in the Introduction, hexachlorobenzene is not included in the chlorinated benzenes category in this document. The chemical structures of the eleven chlorinated benzenes are shown in Figure 1.

B. Category Characteristics

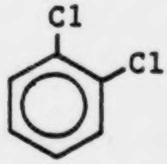
In this document the Agency considers the chlorinated benzenes as a group. However, because most of the studies deal with the compounds individually, for the sake of clarity some of the material presented here is organized separately for monochlorobenzene, dichlorobenzenes, trichlorobenzenes, and so on.

The chlorobenzenes comprise a category of closely related chemical compounds that have been shown to cause or would be expected to cause similar biological consequences upon exposure. The chlorobenzene group is formally constructed by substituting one hydrogen of benzene after another with chlorine, in all possible structural arrangements, resulting in corresponding gradual changes in properties across the series. Proceeding from less chlorinated to more highly chlorinated benzenes, we observe regular changes in characteristics or numerical values over a broad range of categories: chemical and physical properties, method of manufacture, use patterns, nature of impurities, and biological and environmental behavior. Some irregularities do occur within the group that result from different steric and electronic effects among isomers of the same degree of substitution, but these are not significant enough to negate the overall consistency of the group's behavior. For example, _p_-dichlorobenzene is unlike the other two dichlorobenzenes in that it is a

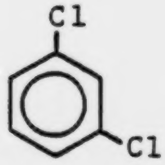
Figure 1. Chemical Structures of the Chlorinated Benzenes



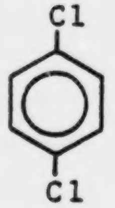
Monochlorobenzene



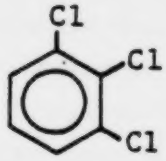
1,2-Dichlorobenzene
o-Dichlorobenzene



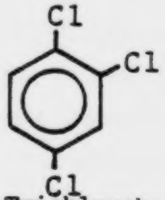
1,3-Dichlorobenzene
m-Dichlorobenzene



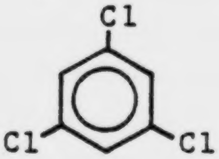
1,4-Dichlorobenzene
p-Dichlorobenzene



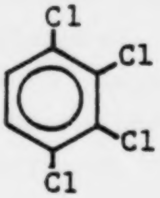
1,2,3-Trichlorobenzene



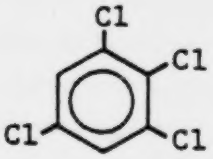
1,2,4-Trichlorobenzene



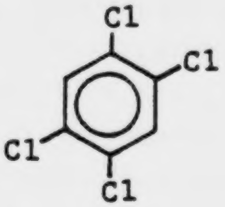
1,3,5-Trichlorobenzene



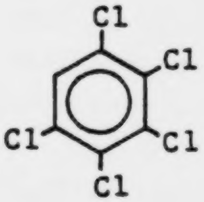
1,2,3,4-Tetrachlorobenzene



1,2,3,5-Tetrachlorobenzene



1,2,4,5-Tetrachlorobenzene



Pentachlorobenzene

solid at room temperature; it is also somewhat less readily chlorinated than the ortho isomer. Yet, like the other dichlorobenzenes, it is more resistant than monochlorobenzene and less resistant than trichlorobenzene to chlorination. Such departures from strict regularity indicate that caution should be exercised in extrapolating biological characteristics from one chlorobenzene to another on the basis of structure, physicochemical properties, and limited test data. However, because of the consistency of the overall trends in physicochemical properties and the similarities in metabolism and health effects discussed in Section III, the EPA believes that once a representative number of compounds has been characterized toxicologically, extrapolation will be possible.

Further, knowledge of the commercial methods for producing and handling the chemicals and the possibility that the chemicals may interconvert to some extent encourage EPA to regard chlorobenzenes as a group. All industrial chlorobenzenes are produced by the chlorination of benzene or of other chlorobenzenes. This practice ensures that most commercial chlorobenzenes will contain other chlorobenzenes as impurities and that chlorobenzene production wastes will also contain various chlorobenzenes. The estimation of relative environmental levels of the various chlorobenzenes is complicated by the possibility that some interconversion of isomers might occur in the environment. This could be the result either of conversion to more highly chlorinated compounds during water treatment by chlorination or of reductive dechlorination by photo-degradative mechanisms or by microorganisms to form the less-chlorinated derivatives. There is little information on this point, although interconversions by dechlorination apparently do occur to some extent during the mammalian metabolism of some chlorinated benzenes (Section III.B.1.c.(1). The potential for human exposure to various chlorobenzenes in unknown proportions thus increases the desirability of focusing on the risks associated with chlorobenzenes as a group.

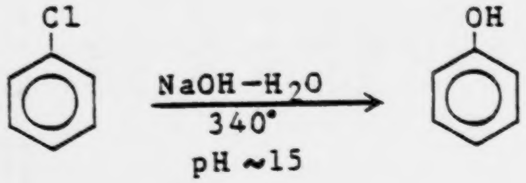
The concern of EPA for those chlorinated benzenes that have lesser or little commercial importance is based on several factors. One is that a seemingly small contaminant in a large quantity of material can represent a sizable contribution to environmental contamination. Thus, the _m_-dichlorobenzene content of commercial _o_-dichlorobenzene ranges from 0.5 percent to at least 2 percent (see Section II.B.1.); if the average content is only one percent, this still represents approximately one-half million pounds annually that may ultimately enter the environment. Furthermore, about the same amount of _m_-dichlorobenzene was imported in 1977 (Section II.B.3), increasing the potential for exposure to the chemical. Both 1,3,5-trichlorobenzene and 1,2,3,5-tetrachlorobenzene are produced as by-products of more commercially important isomers (Section II.C.3 and Section II.D.2,3). The disposition of the 1,3,5-trichlorobenzene was not identified but the 1,2,3,5-tetrachlorobenzene is disposed as waste. Ten thousand pounds of 1,3,5-trichlorobenzene was imported in 1977 (Section II.C.3). The Agency needs more information on the fate of these materials before it can evaluate their potential risk to human health or the environment. Another factor in the concern for the less commercially important chlorinated benzenes is that they as well as the more important category members have been detected in air and water. _m_-Dichlorobenzene has been found in drinking water at a concentration similar to those of the other two dichlorobenzenes (Section II.B.7.). Furthermore, concentrations of airborne _m_-dichlorobenzene near a disposal site exceeded those of the _ortho_ and _para_ isomers. Both 1,3,5-trichlorobenzene and 1,2,3,5-tetrachlorobenzene have been found in fish tissue (Section II.C.7 and Section II.D.7). Both isomers have also been detected in ambient water systems. Hence, even chlorinated benzenes of lesser commercial value have the potential for human exposure.

C. Physical properties

The physical properties of the chlorobenzenes vary in an approximately regular way with increasing substitution. Some of these properties appear in Table I. In general, the chlorinated benzenes have low water solubility, low flammability, moderate to high octanol/water partition coefficients, and low to moderate dielectric constants.

D. Chemical reactivity

Because of the electron-withdrawing character of the chlorine atom relative to carbon, monochlorobenzene is less reactive toward electrophilic attack (e.g., chlorination) than is benzene. Each additional chlorine substituent further lowers the reactivity of the compound. Thus, the more highly substituted chlorobenzenes are expected to be the most stable members of the category toward this type of reaction. When electrophilic substitution does occur on monochlorobenzene, it takes place primarily in the ortho and para positions. Nucleophilic substitution on chlorobenzenes (see equation) is possible under forcing conditions, but in most cases should occur only very slowly, if at all, at environmental temperatures and pH values.



In fact, the reaction shown probably does not proceed by a true substitution mechanism (Roberts and Caserio 1965).

TABLE 1.- PHYSICAL PROPERTIES OF CHLORINATED BENZENES[a]

Compound Name	CAS No.	Empirical formula	Mol. Wt.	M.p., °C	B.p.[b] °C	Vapor Press.	Density[c]	Water Solubility (mg/L)[d]	Log P_{oct}
Monochlorobenzene	108-90-7	C_6H_5Cl	112.56	-45.6°	132°	10mm/22°	1.1058	500[e]	2.84[f]
1,2-Dichlorobenzene	95-50-1	$C_6H_4Cl_2$	147.01	-17.0°	180.5°	1mm/20°	1.3048	140[e]	3.38[f]
1,3-Dichlorobenzene	541-73-1	$C_6H_4Cl_2$	147.01	-24.7°	173°	1mm/12°	1.2884	123[e]	3.38[f]
1,4-Dichlorobenzene	106-46-7	$C_6H_4Cl_2$	147.01	53.1°	174°	0.4mm/25°	1.2475	79[e]	3.39[f]
1,2,3-Trichlorobenzene	87-61-6	$C_6H_3Cl_3$	181.45	54°	219°	1mm/40°			4.1[g,h]
1,2,4-Trichlorobenzene	120-82-1	$C_6H_3Cl_3$	181.45	17°	213.5°	1mm/38°	1.4542	30[i]	
1,3,5-Trichlorobenzene	108-70-3	$C_6H_3Cl_3$	181.45	64°	208°[j]	10mm/78°			
1,2,3,4-Tetrachlorobenzene	634-66-2	$C_6H_2Cl_4$	215.90	47.5°	254°	1mm/68°		0.36[e]	
1,2,3,5-Tetrachlorobenzene	634-90-2	$C_6H_2Cl_4$	215.90	54.5°	246°	1mm/58°			
1,2,4,5-Tetrachlorobenzene	95-94-3	$C_6H_2Cl_4$	215.90	140.5°	246°	40mm/146°	1.858(22°)		
Pentachlorobenzene	608-93-5	C_6HCl_5	250.34	86°	277°	1mm/99°	1.8342(16°)		
Hexachlorobenzene[k]	118-74-1	C_6Cl_6	284.79	230°	322°	1mm/114°	1.5691(24°)	0.020[l]	5.8[g]

[a] Data are from standard reference sources except partition coefficient and solubility data, for which references are cited. [b] At 760 mm. [c] At 20°C except as noted. [d] At 25°C. [e] Verschueren 1977. [f] Leo et al. 1971. [g] Monsanto 1978d. [h] Isomer unspecified. [i] Simmons et al. 1977. [j] At 763 mm. [k] Included for purposes of comparison. [l] Laseter 1976.

II. Exposure Aspects

Note: This section contains information on pesticide uses for several chlorinated benzenes. This information is included in order to present a more complete picture of use patterns rather than to support the case for a test rule under TSCA.

A. Monochlorobenzene

1. Nature of the Substance

At room temperature, monochlorobenzene is a colorless, flammable liquid that is heavier than water (Hawley 1977). According to the Merck Index, it is soluble in alcohol, benzene, chloroform, and ether. Impurities in a reportedly typical analysis were dichlorobenzenes at less than 0.1 percent and benzene at less than 0.05 percent (Kao and Poffenberger 1979), implying a purity of 99.8 percent or higher for the sample. A Dow product data sheet claims 99.9 percent purity for monochlorobenzene (Dow 1977), while Allied states a purity of 99.0 percent for its product (Allied 1973).

2. Manufacture

The manufacture of chlorobenzenes is well described by Hardie (1964) in the Kirk-Othmer Encyclopedia of Chemical Technology. In brief, monochlorobenzene is manufactured by chlorination of benzene at temperatures between 30° and 50°C in the presence of a catalyst. During this process, o-dichlorobenzene, p-dichlorobenzene, and hydrogen chloride are also produced. However, production of the dichlorobenzenes may be minimized by selecting the proper catalyst or an additive such as fuller's earth, and keeping the reaction temperature close to 30°C. The hydrogen chloride is removed by washing with alkali to yield a product with as much as 95 percent monochlorobenzene; subsequent distillation provides a purer product (see II.A.1, above).

3. Production Volume and Trends

According to the 1978 Directory of Chemical Producers (SRI 1978), six companies produce monochlorobenzene in the United States, with a combined annual production capacity of 615 million pounds. Domestic production of monochlorobenzene averaged about 440 million pounds per year during the period 1966 through 1977 (USITC 1966-1977). During this period, production was highest in 1969 at 601 million pounds, and lowest in 1975 at 306 million pounds. In 1976 and 1977, monochlorobenzene production was 329 and 325 million pounds, respectively. In a 1980 report prepared by Hull and Company for the Synthetic Organic Chemicals Manufacturers Association (SOCMA), 1978 production of monochlorobenzene was stated to be 303 million pounds, of which 302 million pounds was reported to be consumed within the United States (Hull 1980).

Imports of monochlorobenzene are small compared to domestic production. Imports were about 1.5 million pounds in 1974 and 8.4 million pounds in 1975 (Allport et al. 1977). According to nonconfidential TSCA Inventory information, five importers reported no imports of monochlorobenzene in 1977 (OPTS 1979a).

4. Uses

Monochlorobenzene consumption has been in a state of flux over the past several years. According to Hardie (1964), the bulk of monochlorobenzene consumption in the early 1960's went to the production of phenol (60 percent) and the insecticide DDT (25 percent), with the remainder used to produce a variety of dye intermediates. However, phenol production from monochlorobenzene was gradually phased out in favor of the cumene process before 1975 (Lowenheim and Moran 1975), while DDT production in the United States dropped from 123 million pounds in 1969 to 54.3 million pounds in 1970 (USITC 1966-1977) as a result of regulations, effective in 1972, severely restricting its sale and use. No production data have been available for DDT since 1970.

The current consumption pattern of monochlorobenzene is not known in detail, but some general information on consumption is available for recent years from three sources. SRI International (Allport et al. 1977) reports that the monochlorobenzene consumption pattern in 1974 was:

Solvent (for pesticides, degreasing)	49%
Chloronitrobenzenes intermediate	30%
Diphenyl oxide intermediate	8%
DDT intermediate	7%
Other uses	6%

The Chemical Marketing Reporter (1977) presented the following consumption pattern:

Solvent	30%
Chloronitrobenzenes intermediate	35%
Diphenyl oxide intermediate	10%
Rubber intermediate*	10%
Synthesis of DDT, silicones, isocyanates, others	15%

Lastly, Dow Chemical (1978b) provided these consumption data:

Chemical intermediate (chloronitrobenzenes, herbicides, diphenyl oxide, DDT, silicones, others)	50-70%
Solvent for herbicide formulations	30-50%
Other (chemical process solvent; degreasing solvent; other uses)	<5%

It appears that the two major categories of monochlorobenzene consumption are as an intermediate and as a solvent. Among its uses as an intermediate, the largest is for the production of o-chloronitrobenzene and p-chloronitrobenzene. Both of these monochlorobenzene derivatives are used as dye intermediates; p-chloronitrobenzene is also used to produce p-nitrophenol.

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*A Dow product bulletin refers to monochlorobenzene's use as a solvent for synthetic rubbers (Dow 1975).

Another derivative, diphenyl oxide (diphenyl ether) is used in perfumery, especially in soaps, and as a heat transfer medium (Hawley 1977, Merck 1976). The silicon derivatives made from monochlorobenzene are phenyltrichlorosilane and diphenyldichlorosilane (Meals 1964), which are used in the production of silicone lubricants (Hawley 1977). No specific information could be found on the other intermediate uses of monochlorobenzene.

There is very little specific information on the use of monochlorobenzene as a solvent. Allied Chemical (1973) indicated in their product data sheet for monochlorobenzene that it has industrial process solvent applications in the manufacture of adhesives, paints, polishes, waxes, diisocyanates, pharmaceuticals, and natural rubber. This source also notes the use of monochlorobenzene as a dye carrier in textile dyeing. The extent of actual use of monochlorobenzene in these areas could not be determined. Dow Chemical (Dow 1978b) stated that the bulk of the solvent use was for unspecified herbicide formulations, with only a small amount of monochlorobenzene going to other solvent uses such as chemical processing or degreasing. Hull and Company (Hull 1980) indicate that monochlorobenzene is used as a "formulation solvent for a consumer auto radiator flush." An EPA (USEPA 1980) survey found monochlorobenzene in industrial water samples from the following industrial classifications (listed in decreasing order of frequency of observation): organics and plastics, petroleum refining, inorganic chemicals, textiles, pesticides, pharmaceuticals, leather, paint and ink, auto and other laundries, printing and publishing, landfill, coal mining, mechanical products, and photographic. This list indicates the variety of possible applications of this substance.

5. Occupational Exposure

Dow Chemical Co. (Dow 1978b) states that the industrial manufacturers and processors of monochlorobenzene employ approximately 10,000 people. The National Occupational Hazard Survey (NIOSH 1979), which includes in addition to manufacturers and processors those potentially exposed on the job to products containing monochlorobenzene, indicates that slightly more than 1 million people may be exposed to monochlorobenzene in the workplace. See Exposure Support Document for a general discussion of the NOHS data.

Hull and Co. estimated that 3,146 people were exposed to monochlorobenzene through its production, its captive use, and receipt of direct shipments from producers (Hull 1980). Several aspects of this report indicate that the worker exposure estimates may be biased toward the low range of exposure (Versar 1980). In any event, the document contains no citations from which the data can be verified (Versar 1980). The survey questionnaire used by Hull and Co. requested best estimates of numbers of people exposed and allowed rounding of these numbers to the nearest 10. Certain key questions relating to workroom exposure due to equipment wear and volatilization during processing were omitted from the form. There was no indication that the data were substantiated by any visits by the survey team or by any means other than follow-up phone calls to the respondents. The numbers of workers exposed do not agree with the worker population identified with the industrial use component of potential exposure to monochlorobenzene (Versar 1980). Finally, the percentage of companies identified as being involved with the manufacture, distribution, and use of monochlorobenzene that responded to the questionnaire was not identified. Consequently, the Hull and Co. figures may represent an underestimate of occupational exposure to chlorinated benzenes.

OSHA (1979) reports two instances in which monochlorobenzene was detected at levels below or equal to the threshold limit value (TLV) in air samples collected from companies using monochlorobenzene. The companies employed 50 and 67 people, respectively. No other information was given. The TLV for monochlorobenzene in workroom air is 75 ppm (350 mg/m^3) (ACGIH 1971).

Pure mono- and dichlorobenzenes tend to be corrosive to metals (Versar 1980). This increases the potential for worker exposure to fugitive emissions from the pump seals, compressor seals, valves and pressure relief devices of the manufacturing machinery (Versar 1980).

No other information was found on occupational exposure to monochlorobenzene in its manufacture, processing or subsequent industrial applications.

6. General Population Exposure

Exposure of the general population to monochlorobenzene appears most likely to occur through release of the compound to the environment through activities such as manufacturing, industrial use, processing and disposal.

7. Environmental Release

Dow Chemical (Dow 1978b) provided an estimate that 30 to 50 percent of the monochlorobenzene produced annually, or 98 million to 162 million pounds, is ultimately released into the air, and less than 0.1 percent (300,000 pounds) into water. The estimated range of losses to the air includes Dow's estimate of annual consumption of monochlorobenzene as a herbicide solvent, a use that would presumably result in virtually complete release to the air. Monsanto (1978c) presented data that showed that direct application of monochlorobenzene to both soil and to plants resulted in a minimum of 80 percent of the monochlorobenzene evaporating within 6 hours after application.

EPA studies have detected monochlorobenzene in air samples collected from the Kin-Buc Land Disposal Site, Edison, N.J. and the air of Birmingham, Alabama (Pellizzari 1978) and in an industrially produced cloud in Henderson, Nevada (Wojinski et al. 1979). Monochlorobenzene levels ranging from 0-12 ug/m^3 (0-2.6 ppb) were detected in the samples from Kin-Buc Land Disposal Site and 38-1000 ng/m^3 (0.008-0.2 ppb) in Birmingham. The Henderson cloud contained 11-40 ug/m^3 (2-8 ppb) of monochlorobenzene, compared to a level of 0.45 ug/m^3 (0.1 ppb) measured in nearby Las Vegas.

In a report by the EPA (Gruber 1975), it was estimated that during the batch manufacture of monochlorobenzene, 0.00088 kg of monochlorobenzene enters the water stream, and 0.004 kg of monochlorobenzene goes to land disposal per kg of monochlorobenzene produced. In 1977, therefore, 130,000 kg (86,000 pounds) would have been released into water and 591,000 kg (1,300,000 pounds) to land; these are maximum figures based on an assumption of 100 percent production by batch processes. Monochlorobenzene was reported to be present in a textile mill effluent (Erisman and Gordon 1975), in an industrial discharge in South Carolina, and in a municipal discharge in Georgia (Ware et al. 1977). Organic priority pollutant surveys conducted by EPA of industrial water and wastewater samples found monochlorobenzene in 147 of 3194 samples with levels ranging from 11 to 6400 ppm (USEPA 1980). The EPA (1975) reported that monochlorobenzene was detected in finished drinking water in 9 out of 10 cities sampled in the National Organics Reconnaissance Survey, with concentrations ranging from 0.1 ug/l to 5.6 ug/l (0.1-5.6 ppb).

Lombardo (1979) reported detection of unspecified concentrations of monochlorobenzene in fresh water fish under the FDA chemical contaminants program.

8. Environmental Transformation

Data from simulated atmospheric photodecomposition experiments indicate that monochlorobenzene may degrade rapidly in the atmosphere. In an experiment performed by Dilling et al. (1976), a half-life of 8.7 hours was found for monochlorobenzene in air. However, the intensity of the ultraviolet light in the test chamber was about 2.6 times that of normal sunlight (as measured at noon on a summer day in Freeport, Texas). No information was presented on the products of monochlorobenzene photodecomposition.

No experimental data were found in the literature on the hydrolysis of monochlorobenzene under environmental conditions. However, this chemical probably will not hydrolyze under environmental pH and temperature conditions (see Section I.D).

In a Monsanto study (1978a), ^{14}C-monochlorobenzene was added to samples of Mississippi River water, and evolved $^{14}CO_2$ was measured over a 63-day period. Twenty-seven percent of the theoretical ^{14}C had been recovered as $^{14}CO_2$ after 63 days. Addition of acclimated activated sludge to a sample of Mississippi River water plus ^{14}C-monochlorobenzene resulted in much slower $^{14}CO_2$ evolution; no explanation was offered for this observation. Gibson et al. (1968) found that the bacterium Pseudomonas putida, growing on toluene, oxidized monochlorobenzene to 3-chlorocatechol. No oxidation or bacterial growth occurred on monochlorobenzene in the absence of toluene, suggesting that biodegradation of the former may proceed relatively slowly.

9. Biological Uptake

The octanol/water partition coefficient of monochlorobenzene is approximately 700 (Leo et al. 1971), suggesting moderate bioconcentration potential. Dow Chemical (Dow 1978b) reported that Japanese experiments indicate a fish bioconcentration factor of less than 300 for monochlorobenzene.

Lu and Metcalf (1975) studied the fate and biomagnification of monochlorobenzene in a model ecosystem using Daphnia, mosquito larvae, fish, snails, and green algae. The results are summarized in Table 1.

Table 1

24-Hour Ecological Magnification[a] (EM) of Monochlorobenzene in Various Aquatic Species

Species	EM[b]	$\frac{^{14}C_6H_5Cl \text{ accumulated}}{\text{Total } ^{14}C \text{ accumulated}} \times 100$
Alga, Oedogonium cardiacum	4185	70
Water flea, Daphnia magna	2789	49
Snail, Physa sp.	1313	53
Mosquito larva, Culex quinquifasciatus	1292	43
Mosquito fish, Gambusia affinis	645	46

[a] Defined as (concentration of compound in organism)/(concentration in water)

[b] Initial concentration = 0.01–0.1 ppm. It appears that the magnifications observed for algae and Daphnia are not maintained at the upper end of the food chain in this test system. It is worth noting that the extent of metabolism of monochlorobenzene, as reflected in the last column of the table, was much less than that found for some other compounds tested under the same conditions. For example, in mosquito larvae 14 percent of anisole and 0 percent of aniline were retained in the form of the unconjugated parent compound, whereas for monochlorobenzene, 43 percent was retained in the unmetabolized form. This order of reactivity conforms with what is expected on chemical grounds and demonstrates the deactivating influence of an aromatic chlorine substituent in a biochemical reaction.

Conclusion: Monochlorobenzene is manufactured on the order of hundreds of millions of pounds annually, more than half of which goes to TSCA uses, primarily as a chemical intermediate. Opportunities for human exposure are both direct, including occupational contact and contact with releases from plants and disposal sites, and indirect, including ingestion via drinking water and, to some extent, the food chain.

B. Dichlorobenzenes

1. Nature of the Substances

At room temperature, *o*-dichlorobenzene and *m*-dichlorobenzene are combustible, neutral, mobile, colorless liquids while *p*-dichlorobenzene is a combustible white crystalline solid that sublimes readily. The dichlorobenzenes are all very soluble in nonpolar solvents (Hawley 1977, Merck 1976) but are poorly water-soluble (see Table 1, Section I.). The lack of industry-wide standards of purity for chlorobenzenes (Kao and Poffenberger 1979) is illustrated by the compositions reported for *o*-dichlorobenzene by different sources as compiled in Table 2.

Table 2

Reported Percentage Compositions of Commercial *o*-Dichlorobenzenes

	Information Source and Percentage Composition					
	Dow (1977)	Allied (1973)	MCA (1974)		Kao and Poffenberger (1979)	
Constituent		Standard Grade	Mechanical Grade	High Purity Grade	Tech. Grade	Purified Grade
C_6H_5Cl		0.07			<0.05	<0.05
o-$C_6H_4Cl_2$	80	82.7	75-85	99.0	80	98
m-$C_6H_4Cl_2$	2	0.5	0.5	"balance"	19	
p-$C_6H_4Cl_2$	17	15.4	15-25			
$C_6H_3Cl_3$ (all isomers)		1.6				<1
1,2,4-$C_6H_3Cl_3$						<0.02

The commercially available technical grade of p-dichlorobenzene contains 0.08 percent by weight of the meta and ortho isomers of dichlorobenzene, but may also contain monochlorobenzene and trichlorobenzenes (IARC 1974, Brown et al. 1975, Kao and Poffenberger 1979). A Dow product data sheet (1977) states a purity of 99.95% for that company's p-dichlorobenzene. The para isomer is commercially available in either the liquid or the solid form (Anon. 1979a). Product information from Montrose Chemical (1972) describes a mixture of 35 percent o- and 65 percent p-dichlorobenzene. Contaminants of m-dichlorobenzene are the ortho and para isomers in unspecified proportions (Hardie 1964).

2. Manufacture

o-Dichlorobenzene and p-dichlorobenzene are produced by chlorinating benzene or monochlorobenzene at 150°-190°C over a ferric chloride ($FeCl_3$) catalyst. An orienting catalyst such as benzenesulfonic acid or p-methylbenzenesulfonic acid will increase yields of p-dichlorobenzene. The isomers can be separated by fractional distillation, or by crystallizing the p-dichlorobenzene. Another method of obtaining the para isomer is by chlorination of crude dichlorobenzene over $FeCl_3$, whereby the more reactive ortho isomer is converted to 1,2,4-trichlorobenzene. p-Dichlorobenzene can then be separated by distillation. Instead of chlorination, selective sulfonation of the ortho isomer with chlorosulfonic acid allows a similar separation. The purified grade of o-dichlorobenzene is obtained by efficient redistillation of the technical product (Kao and Poffenberger 1979). m-Dichlorobenzene can be prepared by isomerization of o-dichlorobenzene and p-dichlorobenzene with heat under pressure in the presence of a ratalyst (Hardie 1964).

3. Production Volume and Trends

TSCA Inventory data indicate that in the United States in 1977 there were eight manufacturers of o- and p-dichlorobenzene, and five manufacturers of m-dichlorobenzene (OPTS 1979a). The total combined production capacity for o-dichlorobenzene and p-dichlorobenzene was 279 million pounds per year in 1978 (SRI 1978) and 307 million pounds per year in 1979 (Anon. 1979a,b). The Dow Chemical Company reports that m-dichlorobenzene is produced as a by-product in the manufacture of other chlorinated benzenes. It is separated from the ortho and para isomers, and further chlorinated to tri- and tetrachlorobenzenes. Only very small quantities are produced for research purposes (Dow 1978b). The EPA estimates on the basis of TSCA Inventory information that 666,000 pounds of the meta isomer was produced in 1977; some of this production was site-limited (OPTS 1979a).

An average of 60 million pounds of o-dichlorobenzene per year was produced from 1966 through 1973. In 1976 the amounts of o-dichlorobenzene and p-dichlorobenzene produced annually were 48.6 million pounds and 36.7 million pounds, respectively. The average sales for o-dichlorobenzene from 1966 through 1975 was 52 million pounds per year. The industry expects only very slow growth in the market for o-dichlorobenzene through 1983 because almost all end-use sectors are mature (Anon. 1979a). In 1978, demand equalled 55 million pounds, and in 1979, it was projected at 56 million pounds (Anon. 1979b). Hull (1980) reports that 59 million pounds of o-dichlorobenzene was produced and that domestic sales and captive use accounted for 52.5 million pounds in 1978. p-Dichlorobenzene sales averaged 67 million pounds per year from 1966 through 1973. During 1975, p-dichlorobenzene sales dropped to a 10-year low of 34 million pounds (USITC 1976). By 1978, demand was back up to 68 million pounds and a projected 67 million pounds in 1979. Hull (1980) estimated 1978 production of p-dichlorobenzene to be 72.5 million pounds and domestic consumption to be 53.2 million pounds. A market decline of two to three percent per year is expected through 1983 because space odorant and mothproofing markets, which comprise 80 percent of para-dichlorobenzene uses, are essentially mature. In addition, rising production costs, the appearance of cheaper substitutes, and the popularity of synthetic fabrics which require no mothproofing, will combine to decrease production and sales volumes until 1983. However, a new use, as an intermediate in the production of the resin polyphenylene sulfide, may help maintain demand. EPA TSCA Inventory-based estimates of 1977 imports of o-, m-, and p-dichlorobenzene are 2 thousand, 551 thousand, and 56 thousand pounds, respectively (OPTS 1979a). Exports of dichlorobenzenes for the months of June and July, 1979 totaled 4.39 million pounds (Anon. 1979c).

4. Uses

Sixty-five percent of the o-dichlorobenzene produced goes to organic synthesis, primarily as a pesticide intermediate. Fifteen percent is used as a solvent in the production of toluene diisocyanate (Anon. 1979b). Miscellaneous solvent uses, such as for oxides of nonferrous metals (Hawley 1977), for soft carbon deposits, for tars and wool oils in the textile industry (Dow 1975) and for degreasing leather and automobile and aircraft engine parts (Allied 1973), account for most of the rest of the annual production of o-dichlorobenzene. It is also a solvent in formulated toilet bowl cleaners and drain cleaners (Allied 1973). Other uses in metal polishes, in industrial odor control, as a heat transfer fluid, and in rustproofing mixtures account for 4 percent of annual o-dichlorobenzene production (Lowenheim and Moran 1975). o-Dichlorobenzene is registered as a fumigant and insecticide against termites, beetles, bacteria, slime, and fungi (Hawley 1977). Dow Chemical indicated that o-dichlorobenzene is used in the following ways (Dow 1978b).

Chemical Intermediate (herbicides, other)	70-75%
Process Solvent (toluene diisocyanate)	15-20%
Miscellaneous (solvent, heat transfer fluid, other)	5-10%

Eighty percent of the annual production of p-dichlorobenzene goes to home and industrial use as a moth control agent (30 percent) and as a space odorant (50 percent), especially in toilets and rest rooms.

Miscellaneous uses as a dye intermediate, insecticide, extreme pressure lubricant, forming agent for grinding wheels, disintegrating paste for molding concrete and stoneware, and as an intermediate in the manufacture of 2,5-dichloroaniline and polyphenylenesulfide resins account for 10 to 20 percent of the consumption of p-dichlorobenzene (Allied 1973, Dow 1975, Anon. 1979a). No uses were identified for m-dichlorobenzene apart from its conversion to higher chlorobenzenes (Section II.B.3).

In an EPA industrial wastewater survey (USEPA 1980) the industrial categories with dichlorobenzenes present (listed in decreasing order of frequency of occurance) were: organics and plastics, steam and electric, leather, textiles, auto and other laundries, mechanical products, printing and publishing, pesticides, nonferrous metals, electrical, pharmaceutical, adhesives and sealants.

5. Occupational Exposure

Occupational exposure to o-dichlorobenzene can occur through the inhalation of vapors during its production, processing, and use as an industrial solvent and in commercial products. The potentially occupationally exposed population figure given by the National Occupational Hazard Survey (NOHS) for all occupational activities involving o-dichlorobenzene is approximately 2 million (NIOSH 1979); Dow estimates that 10,000 workers are potentially occupationally exposed as a result of production, processing and industrial solvent use (Dow 1978b). For p-dichlorobenzene, the NIOHS estimate for potential worker exposure during its production, processing into space odorants, and use as an intermediate is approximately 1 million (NIOSH 1979). Hull (1980) estimated that 1,311 people were exposed to o-dichlorobenzene and 821 people were exposed to p-dichlorobenzene during production, captive use, and shipment from producers. Several aspects of this report indicate that the worker exposure estimates may be biased toward the low range of exposure (Versar 1980). In any event, the document contains no citations from which the data can be verified (Versar 1980). The survey questionnaire used by Hull and Co. requested best estimates of numbers to the nearest 10. Certain key questions relating to workroom exposure due to equipment wear and volatilization during processing were omitted from the form. There was no indication that the data were substantiated by any visits by the survey team or by any means other than follow-up phone calls to the respondents. The numbers of workers exposed do not agree with the worker population identified with the industrial use component of potential exposure to dichlorobenzenes (Versar 1980). Finally, the percentage of companies identified as being involved with the manufacture, distribution, and use of dichlorobenzenes that responded to the questionnaire was not identified. Consequently, the Hull and Co. figures may represent an underestimate of occupational exposure to chlorinated benzenes.

No information was found on the number of workers exposed to _m_-dichlorobenzene. Between 1973 and March of 1979, OSHA inspectors collected, from a total of 15 companies throughout the United States, air samples that contained dichlorobenzenes at levels less than or equal to the TLV standards in effect at the time of sampling. A total of 8000 people were involved. The air samples from nine companies (6750 people) contained _o_-dichlorobenzene; samples from four companies contained _p_-dichlorobenzene (400 people), and samples from two companies (1800 people) contained both isomers (USOSHA 1979). The TLV's for _o_- and _p_-dichlorobenzene are 50 ppm and 75 ppm, respectively (ACGIH 1978). No TLV has been established for _m_-dichlorobenzene.

A 1940 air-monitoring study in a Dow _p_-dichlorobenzene plant found concentrations in working areas ranging from 12 ppm to 550 ppm; more than half the values were in the range 50-175 ppm. Insufficient details were available to determine whether the measurements were selective for the para isomer or whether other chlorinated benzenes might have been included in the figures reported. Some of the exposed workers had developed a local dermatitis (Dow 1978c).

Another study (Pagnotto and Walkley 1965) reported levels of dichlorobenzenes in a chlorobenzenes manufacturing plant, in a factory where p-dichlorobenzene was processed into moth cakes, and in a plant where p-dichlorobenzene was used in an abrasive wheel-forming process. Average levels of p-dichlorobenzene for various working areas ranged from 24-34 ppm in the chlorobenzenes plant (high value 49 ppm), from 9-25 ppm in the moth cake operation (high value 34 ppm), and from 8-11.5 ppm in the abrasive wheel facility (high value 14.5 ppm). The authors also referred to workers in the moth cake plant being struck occasionally by a fine dust burst from a p-dichlorobenzene pulverizing machine. In the chlorobenzenes plant, o-dichlorobenzene was detected at levels up to 25 percent of those for the para isomer. A metabolite of p-dichlorobenzene, 2,5-dichlorophenol, was found in the urine of exposed workers; although metabolite excretion dropped off rapidly when exposure was terminated, it was still in evidence after several days, suggesting that some retention of p-dichlorobenzene or its metabolites occurs after exposure.

Hull and Co. (Hull 1980) indicate that the major use of o-dichlorobenzene in formulated products is as an industrial cleaner. Over 4 million pounds of o-dichlorobenzene went into degreasing and decarbonizing formulations for use in the automotive, trucking, and aircraft industries. During the use of these formulations, workers are exposed to fumes throughout the entire working day. Hull (1980) indicates that 200 workers may be exposed in transmission shops alone.

6. General Population Exposure

General population exposure to o-dichlorobenzene and p-dichlorobenzene occurs because the chemicals are present in many household products. Sources of consumer exposure to o-dichlorobenzene are auto engine parts cleaners, toilet bowl cleaners, and drain cleaners. Household exposure to p-dichlorobenzene occurs because of its use in deodorizers (Hull 1980). Morita and Ohi (1975) reported indoor concentrations of p-dichlorobenzene from its use as a space odorizer or moth repellant of 1700 ug/m^3 (283 ppb) (inside wardrobe); 315 ug/m^3 (52 ppb) (closet); and 105 ug/m^3 (18 ppb) (bedroom). Further exposure to dichlorobenzenes may occur by way of the environment (see Environmental Release, below).

7. Environmental Release

Because o-dichlorobenzene is relatively volatile and has widespread use in industry as a solvent and as an intermediate in the manufacture of other chemicals, release to the atmosphere may occur readily. Because p-dichlorobenzene readily sublimes and is used extensively as a domestic and industrial space deodorant, extensive quantities are released into the atmosphere.

Dow Chemical's estimates for the release of the dichlorobenzenes into the environment as a percentage of their annual production are 5 to 10 percent in air (2.4 to 4.8 million pounds based on 1976 production),and less than 0.1 percent (0.49 million pounds) in water for o-dichlorobenzene, and 70 to 90 percent (26 to 33 million pounds) in air and less than 5 percent (1.8 million pounds) in water for p-dichlorobenzene (Dow 1978b). Other estimates of the environmental release rates are 43 percent (21 million pounds) for o-dichlorobenzene and 91 percent (34 million pounds) for p-dichlorobenzene (calculated from data in Allport et al. 1977). No release rate was found for m-dichlorobenzene.

o-Dichlorobenzene was reported to be lost to the environment during manufacture at the rate of 0.9 million pounds per year in 1972. These losses arose through venting and scrubber washings (Brown et al. 1975). When used as a solvent in the manufacture of toluene diisocyanate (TDI), o-dichlorobenzene is lost to the environment through venting, scrubbers, and leaking equipment; the sludge and hydrogen chloride remaining after TDI manufacture contain less than a few hundred parts per million of o-dichlorobenzene (Brown et al. 1975). Environmental losses during manufacture for p-dichlorobenzene were estimated to be 1.2 million pounds in 1972 (Brown et al. 1975). Dow Chemical estimates that between 73 and 99 percent of the p-dichlorobenzene used in deodorant blocks evaporates into the air (Dow 1978b).

Dichlorobenzenes also are lost to the environment as a result of monochlorobenzene manufacture. According to an EPA report (USEPA 1975), the loss of dichlorobenzenes to waste waters from this source is 0.37 percent of monochlorobenzene production, or 1.2 million pounds per year on a 1977 basis; land disposal amounts to 0.01 percent, or 32,500 pounds in 1977.

Dichlorobenzenes have been detected in air samples from the Kin-Buc Disposal Site Edison, N.J., Birmingham, Ala. the Chambers Works site of the E.I. DuPont de Nemours Co, at Deepwater, N.J. (Pellizzari 1978) and in an industrial cloud which periodically develops over Henderson, Nevada (Wojinski et al. 1979). All three isomers of dichlorobenzene were detected within a 5-mile radius of the Edison, N.J. disposal site. o-Dichlorobenzene concentrations ranged from 0-10 ug/m^3 (0-2 ppb); p-dichlorobenzene from 0-0.5 ug/m^3 (0-0.08 ppb); and m-dichlorobenzene from 0.2-33 ug/m^3 (0.03-5.5 ppb). o-Dichlorobenzene was also detected at the Chambers Works site (1.319 ug/m^3) and in Birmingham (0.348 ug/m^3). m-Dichlorobenzene was detected in Birmingham (0.258-0.557 ug/m^3) (Pellizzari 1978). The Henderson cloud contained a total of dichlorobenzene isomers of 1.6-33 ug/m^3 (0.3-5.5 ppb) compared to a range of 0-6.5 ug/m^3 (0-1.1 ppb) found in nearby Las Vegas (Wojinski et al. 1979).

p-Dichlorobenzene used in toilet blocks is flushed into sewer systems. A Dow study (1978d) found that p-dichlorobenzene urinal blocks, when subjected to intermittent water washes to simulate flushing conditions, dissolved to the extent of 0.26-0.67 mg/L on each washing. Continuous dropwise washing dissolved up to 18 mg/L. The solvent effect of urine was not investigated. p-Dichlorobenzene also has been detected in textile finishing plant effluents (Erisman and Gordon 1975). EPA monitoring data have indicated the presence of o-dichlorobenzene and p-dichlorobenzene in industrial discharges.

Young and Heesen (1977) examined the effluent from the Los Angeles County, Los Angeles City, and Orange County sewage treatment plants along the Southern California Bight. Both _p_-dichlorobenzene and _o_-dichlorobenzene were detected in the water samples. Values were generally in the range of 2-12 ug/L, although levels as high as 230 ug/L (para isomer) and 435 ug/L (ortho isomer) were reported for samples from the Los Angeles City treatment plant effluent. None of the three treatment plants treated their sewage by chlorination. Sediments collected from the vicinity of the Los Angeles County sewage plants contained detectable levels of both dichlorobenzene isomers as did the muscle and liver tissue of fish collected from the same area. Up to 0.010 mg/L of an unspecified isomer of dichlorobenzene was detected in cooling water and in seepage lagoons at a detergent manufacturing plant in Muskegon, Michigan (Christensen and Long 1976). Organic priority pollutant surveys conducted by EPA of industrial water and wastewater samples found all three dichlorobenzene isomers in 178 out of 3,268 samples at levels greater than 10 ppb (USEPA 1980). As the isomers were difficult to determine by the procedures used, the three isomers were combined for frequency of occurance analysis. Samples collected from the wastewater treatment plants of Dalton, Calhoun, and Rome, Georgia and from the Coosa River contained from 0.004 mg/L to 0.268 mg/L of unspecified dichlorobenzene. Concentrations of the dichlorobenzenes tended to increase in the downstream direction and in some cases were higher in the finished water than in the treatment plant water. The Coosa River is the center of the tufted carpet and rug industy (Gaffney 1976). A survey of environmental monitoring data on the occurrence of volatile organics in drinking water indicated that _p_-dichlorobenzene was present in 12.5% of the finished drinking water and in 12.9% of the finished ground water samples from stations reporting usable data. In a New Jersey study, 7 out of 717 samples contained detectable levels of unspecified dichlorobenzenes (Coniglio et al. 1980). The highest concentrations of _o_-dichlorobenzene, _m_-dichlorobenzene, and _p_-dichlorobenzene found in drinking water were 0.0010 mg/L, 0.0005 mg/L, and 0.0005 mg/L, respectively (EPA 1975). The fact that maximum levels are similar for the three isomers is of interest in view of the low production volume of the meta isomer relative to the other two dichlorobenzenes. Fish specimens collected from the Great Lakes drainage system have had detectable levels of dichlorobenzenes in their tissue (Veith et al. 1979), as did those from other fresh water sources (Lombardo 1979).

The dichlorobenzenes can volatilize from water. Some laboratory data indicate that these compounds volatilize nearly completely from nonaerated distilled water in less than three days using initial concentrations of 100 mg/L for o-dichlorobenzene and 300 mg/L for p-dichlorobenzene (Garrison and Hill 1972).

8. Environmental Transformation

No experimental data have been found on the environmental hydrolysis of dichlorobenzenes. However, the dichlorobenzenes are probably not readily susceptible to hydrolysis under environmental pH and temperature conditions (see Section I.B.2.). In the atmosphere, o- and p-dichlorobenzene react with hydroxyl radicals and have a half-life of 3 days (Brown et al. 1975). No data were found on the rates of photolytic decomposition in the atmosphere.

A study done for Monsanto (1978b) showed that both o- and p-dichlorobenzene are biodegradable by acclimated microorganisms. The total biodegradation time (determined from 27-day CO_2 evolution rates) was calculated to be 55 days for o-dichlorobenzene and 58 days for p-dichlorobenzene. A Dow study (1978d) showed that acclimated activated sludge can remove 95 percent of p-dichlorobenzene from the water within 7 days.

9. Biological Uptake

The octanol/water partition coefficient for both o-dichlorobenzene and p-dichlorobenzene is 2400 (calculated from Table 1, Section I), suggesting some bioconcentration potential. Dow found bioconcentration factors for p-dichlorobenzene of 231 for trout and 15 for bluegill. After 10 weeks' exposure to p-dichlorobenzene, the bioconcentrated compound was not detected in bluegills within one week after they were transferred to clean water (Dow 1978a); analogous data were not reported for the trout.

Conclusion: About 100 million pounds of dichlorobenzenes is produced each year. They have chemical intermediate, space odorant, and solvent uses. Considerable loss to the environment occurs during their manufacture, processing, and use and as a result of their formation during monochlorobenzene manufacture. Potential for exposure to dichlorobenzenes results from occupational contact during manufacture and industrial use, from consumer product use, and from consumption of drinking water and fish, and also occurs as a result of disposal.

C. Trichlorobenzenes

1. Nature of the Substances

At room temperature, 1,2,4-trichlorobenzene is a colorless liquid while 1,2,3- and 1,3,5-trichlorobenzene are crystalline solids (Hardie 1964). All are soluble in ethanol (Merck 1976) and have low water solubility (see Table 1, Section I.). Commercial 97 percent 1,2,4-trichlorobenzene may contain mono-, di- and tetrachlorobenzenes (Kao and Poffenberger 1979). One company is reported to produce both pure and technical grades of 1,2,4-trichlorobenzene, with no further details given (Anon. 1979d). A Dow Chemical information sheet states a purity of 100 percent for that company's 1,2,4-trichlorobenzene (Dow 1977). An entry on the TSCA Inventory, Pyranol 1478, is identified only as trichlorobenzene (USEPA 1979a).

2. Manufacture

1,2,4-Trichlorobenzene is produced along with 1,2,3-trichlorobenzene by chlorination of o-dichlorobenzene at 25°-30°C in the presence of ferric chloride, then separated from the 1,2,3-trichlorobenzene by distillation (Hardie 1964). 1,3,5-Trichlorobenzene can be produced readily only by special methods such as by the diazotization of 2,4,6-trichloroaniline followed by treatment with alcohol (Merck 1976). Additional methods of synthesis for trichlorobenzenes are reviewed in the report by Ware and West (1977).

3. Production Volume and Trends

Production of 1,2,4- and 1,2,3-trichlorobenzene, and mixtures of the two, increased from 9.3 million pounds in 1970 to 28.3 million pounds in 1973 (USITC 1966-1977). International Trade Commission production figures for the trichlorobenzenes have not been available since 1973. In that year, 1,2,4-trichlorobenzene was produced by four manufacturers in the United States. Since 1974, according to the International Trade Commission there have been only two manufacturers of 1,2,4-trichlorobenzene (USITC 1966-1977); the TSCA Inventory lists four for 1977 (OPTS 1979a). Dow (1979) estimated the annual domestic production at less than 20 million pounds. There is one manufacturer of a mixture of 1,2,3- and 1,2,4-trichlorobenzene (Dow 1979). Imports for 1977 of the 1,2,4-isomer are estimated at around one million pounds by EPA using Inventory data (OPTS 1979b).

Most trichlorobenzene produced in the United States for commercial purposes consists of the 1,2,4-isomer. In the production of the 1,2,4-isomer, 1,2,3-trichlorobenzene is also produced, but can be separated from the 1,2,4-isomer by distillation. A very small quantity of 1,3,5-trichlorobenzene is also produced as a by-product in this process, but according to Dow Chemical, this quantity amounts to less than 6,000 pounds per year (Dow 1979). Nevertheless, TSCA Inventory data indicate that at least 20 thousand pounds of 1,3,5-trichlorobenzene was produced in 1977 by four manufacturers and that a fifth either produced or imported at least 10 thousand pounds. Production and imports of the 1,2,3-isomer were both in the one to ten million pound range for 1977 (OPTS 1979b). Dow's estimate for 1,2,3-trichlorobenzene production is four million pounds per year maximum (Dow 1979).

4. Uses

The major uses of 1,2,4-trichlorobenzene are as a dye carrier (20-30 percent), a synthesis intermediate (50-60 percent), as a dielectric fluid (5-10 percent), and as a solvent (5-10 percent) (Dow 1979). As a dye carrier, 1,2,4-trichlorobenzene is used to facilitate the dyeing of textiles (Jones 1973). A carrier swells the fibers of a material and allows the dye to diffuse through the fiber more quickly. Upon completion of the dyeing process, the dye carrier is removed from the fabric and disposed (Mark 1966). As an herbicide intermediate, 1,2,4-trichlorobenzene is used to manufacture 2,5-dichlorobenzoic acid (Dow 1975). Other uses of 1,2,4-trichlorobenzene are as a solvent for coal tar products such as asphalt, for oil-soluble dyes, and for wood preservatives (Dow 1975); its use in lubricants has also been cited (Hawley 1977). The quantities of 1,2,4-trichlorobenzene utilized in each case are not known. Its high thermal conductivity and heat capacity along with its anticorrosion characteristics make 1,2,4-trichlorobenzene useful as a heat transfer fluid. It is also used as an insulating (dielectric) fluid in transformers and capacitors (Dow 1975, 1979) and is thus a potential substitute for PCB's; at least one U.S. company plans to import such a product, Iralec©, from a French manufacturer (Prodolec 1979). For all except chemical intermediate uses, the uses of 1,2,3- and 1,2,4-trichlorobenzene are the same, and mixtures of the two can be employed (Dow 1979). For example, a sample of transformer fluid involved in a spill proved to contain 28% 1,2,4-trichlorobenzene and 4% 1,2,3-trichlorobenzene by weight (Lewis 1979). No data were found on uses for 1,3,5-trichlorobenzene other than its possible use as a starting material for tetrachlorobenzenes.

5. Occupational Exposure

NIOSH has estimated that 86,340 people are potentially exposed to 1,2,4-trichlorobenzene in the workplace each year and that about 1,867 are exposed to 1,3,5-trichlorobenzene* (NIOSH 1979). Dow estimates that fewer than 12,500 persons are exposed to trichlorobenzenes used as dye carriers, less than 2,500 as a result of dielectric applications, and less than 300 in connection with synthetic chemical production (Dow 1979). No estimate was provided for exposure resulting from miscellaneous solvent uses. The TLV for 1,2,4-trichlorobenzene is 5 ppm (ACGIH 1971). No TLVs for the other two trichlorobenzene isomers have been established.

6. General Population Exposure

The uses of trichlorobenzenes are such that any general population exposure is most likely to occur as a result of their release to the environment - e.g. by disposal or leakage of dielectric fluid.

7. Environmental Release

In a data review by Coniglio et al. (1980), 11.5% of the finished ground water stations surveyed for volatile organic substances had 1,2,4-trichlorobenzene present in the water. A New Jersey ground water survey covered in the same review found trichlorobenzene present in 13 of 396 samples. 1,3,5-Trichlorobenzene has been detected in wastewater discharges in Southern California (Young and Heeson 1978). 1,2,4-Trichlorobenzene has been found in textile plant effluents (Erisman and Gordon 1975). Organic priority pollutant surveys conducted by EPA of industrial water and wastewater samples found 1,2,4- trichlorobenzene at levels greater than 10 ppb in 30 out of 3266 samples (EPA 1980). The water levels ranged from 12 to 607 ppb from the following industrial classes (listed in decreasing order of occurrence): textiles, organics and plastics, foundries, nonferrous metals, pesticides, electrical, and paint and ink. Trichlorobenzenes have also been detected in both waste water treatment plant and Coosa River water samples in Georgia (Gaffney 1976). Garrison and Hill (1972) indicated that 1,2,4-trichlorobenzene at a concentration of 100 mg/L volatilized from aerated water in less than 4 hours and from unaerated water in less than 48 hours. Thus, initial entry of this isomer into water may in some situations lead to fairly rapid transfer of most of the substance to the atmosphere (but see part 8 below).

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* EPA is attempting to confirm that the 1,3,5- isomer and not the higher-production 1,2,3- isomer is intended.

Tissues of freshwater fish from the Great Lakes and the Arkansas River (Veith et al. 1979) and from other locations (Lombardo 1979) have been found to contain unspecified amounts of trichlorobenzenes Muscle and liver tissue of Dover sole collected from the Southern California Bight contained detectable levels of 1,2,4- and 1,3,5-trichlorobenzene (Young and Heeson 1977).

8. Environmental Transformation

Few data have been published on the physical and chemical properties of the trichlorobenzenes that govern their transport and transformation in the environment.

1,2,4-Trichlorobenzene is slowly biodegradable. Experiments by Simmons et al. (1977) showed that a residence time in excess of five days in a wastewater treatment plant may be required to significantly reduce its concentration. The relatively small amount that was not degraded in an activated sludge system was mostly bound to the waste sludge (Simmons et al. 1977). In an aerated mixed aerobic microbial culture, 1,2,4-trichlorobenzene was still detectable after 9 days (Garrison and Hill 1972). From this information and that in part 7 above, it appears that 1,2,4-trichlorobenzene in water is subject to both evaporation and microbial degradation, but that both processes may be retarded by the presence of undissolved matter.

9. Biological Uptake

Although no experimental data are available on the bioconcentration potential of trichlorobenzenes, the reported octanol/water partition coefficient (log P_{oct} = 4.1 - see Table

1, Section I) implies a potential for bioaccumulation.

Conclusion: Trichlorobenzene production and importation total around 20 million pounds yearly. They are used principally as synthesis intermediates, dye carriers, solvents, and dielectric fluid components. People may be exposed to trichlorobenzenes occupationally during their manufacture, processing, and use, and indirectly by way of drinking water and fish consumption. The extent of occurrence of trichlorobenzenes in consumer products is poorly characterized but there may be some direct exposure from this source. Because trichlorobenzenes occur in some commercial dichlorobenzenes, there is additional exposure potential as a result of activities involving the latter. The physicochemical properties of trichlorobenzenes imply some potential for bioaccumulation.

D. Tetrachlorobenzenes

1. Nature of the Substances

All three of the tetrachlorobenzenes occur as white needles that are soluble in most organic solvents and slightly soluble in alcohol (Hardie 1964). A commercial 1,2,4,5-tetrachlorobenzene was analyzed as 97.0 percent pure; impurities were not identified (Kao and Poffenberger 1979, Dow 1977).

2. Manufacture

1,2,3,4-Tetrachlorobenzene can be produced by chlorinating 1,2,3-trichlorobenzene in the presence of a catalyst. 1,2,4,5-Tetrachlorobenzene is manufactured by chlorinating 1,2,4-trichlorobenzene over aluminum amalgam. To produce 1,2,3,5-tetrachlorobenzene, 1,3,5-trichlorobenzene can be chlorinated over aluminum amalgam (Hardie 1964). In practice, 1,2,3,4- and 1,2,3,5-tetrachlorobenzene are produced only as by-products in the manufacture of 1,2,4,5-tetrachlorobenzene (Dow 1979).

3. Production Volume and Trends

According to the U.S. International Trade Commission, there is only one producer of 1,2,4,5-tetrachlorobenzene in the United

States. In 1973, consumption of the material reportedly amounted to approximately 18 million pounds (USITC 1969-1977). However, the TSCA Inventory data (OPTS 1979b) indicate that two manufacturers produced 1,2,4,5-tetrachlorobenzene in 1977, with one reporting a production volume range of 10 to 50 million pounds; the other reported site-limited production. The Inventory includes three companies importing the chemical in 1977, with one reporting 1977 imports of 1 to 10 million pounds. Dow (1979) estimated U.S. production of 1,2,4,5-tetrachlorobenzene as less than 12 million pounds/year.

1,2,3,4- and 1,2,3,5-Tetrachlorobenzene are formed as byproducts during the production of 1,2,4,5-tetrachlorobenzene. The TSCA Inventory data (OPTS 1979b) indicate that there was one active manufacturer of 1,2,3,4-tetrachlorobenzene in 1977. Dow (1979) estimates that the annual production volume of the 1,2,3,4-isomer is about 66 percent of 1,2,4,5-tetrachlorobenzene production; this would be less than 12 million pounds on the basis of both 1973 consumption and a Dow estimate of current production (Dow 1979). Dow also states that the 1,2,3,5-isomer formed during the production of 1,2,4,5-tetrachlorobenzene is separated by distillation and disposed of as waste (Dow 1979). The method of disposal was not given.

Tetrachlorobenzenes are found in some commercial trichlorobenzenes. A sample of transformer fluid was found to contain 16% by weight of two tetrachlorobenzene isomers (Lewis 1979) that were not further identified.

4. Uses

The most widely used isomer of tetrachlorobenzene is 1,2,4,5-tetrachlorobenzene, which is used primarily as an intermediate in chemical synthesis (Dow 1979). Of the approximately 18 million pounds consumed in 1973, six million pounds went to produce the fungicide and bactericide 2,4,5-trichlorophenol, 10 million pounds went to the production of the herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), and the remaining 2 million pounds went to miscellaneous uses. 1,2,4,5-

Tetrachlorobenzene may also be used as an impregnant for moisture resistance, as electrical insulation and as temporary packing protection (Hawley 1977). According to a recent review, 1,2,4,5-tetrachlorobenzene is now used exclusively to make 2,4,5-T and its esters (Kao and Poffenberger 1979). However, 1,2,4,5-tetrachlorobenzene is a component of the transformer fluid, Iralec©, referred to in Section II.C.4. Thus, the use pattern for this material appears to be in flux. EPA has no information on uses of 1,2,3,4- and 1,2,3,5- tetrachlorobenzenes except that the former, as a mixture with the 1,2,4,5-isomer, is an intermediate in the synthesis of the fungicide pentachloronitrobenzene (Dow 1979).

5. Occupational Exposure

According to Dow Chemical (1979), manufacture and use of tetrachlorobenzenes are conducted in closed systems, and fewer than 200 people are occupationally exposed to them. There are no TLVs established for the tetrachlorobenzenes. Occupational exposure to trichlorobenzenes (Section II.C.5) may also involve exposure to tetrachlorobenzenes present as impurities.

6. General Population Exposure

General population exposure to tetrachlorobenzenes appears most likely to occur as a result of their release to the environment, through activities such as manufacturing, industrial use, processing and disposal.

7. Environmental Release

The extent to which tetrachlorobenzenes enter the environment is not known. Small amounts of 1,2,3,4-tetrachlorobenzene and 1,2,3,5-tetrachlorobenzene have been found in fish taken from the vicinity of a kraft pulp and paper mill discharge in Nipigon Bay, Lake Superior (Kaiser 1977). This may be the result of discharges from the chlorine bleaching process used to lighten the color of the pulp. Tetrachlorobenzenes have also been found in fish from the Saginaw (Michigan) and Ashtabula (Ohio) Rivers

(Veith et al. 1979) and other fresh water locations (Lombardo 1979). The exact amounts of the tetrachlorobenzenes found were not determined in these studies. Levels of tetrachlorobenzenes ranging up to 42 ppb have been found in herring gull eggs from several sampling areas in the Great Lakes region (International Joint Commission 1979).

8. Environmental Transformation

No information was found on the environmental transformation of tetrachlorobenzenes. On the basis of their known and expected physicochemical properties, they could be expected to exceed trichlorobenzenes in persistence in the environment. Thus, tetrachlorobenzenes were still readily detectable in roadside soil samples seven months after a spill of used transformer fluid has occurred. Although some loss of tetrachlorobenzenes had occurred (presumably by evaporation), particularly from the top inch of affected soil, 43% of the original tetrachlorobenzenes was present in the next lower inch (Lewis 1979).

9. Biological Uptake

There are no experimental data on the bioconcentration potential of tetrachlorobenzenes. They can be expected to have some bioaccumulation potential, and on the basis of their known and expected physicochemical properties probably exceed the trichlorobenzenes in this respect.

Conclusion: About 20 million pounds per year of tetrachlorobenzenes is produced as a chemical synthesis intermediate, as a dielectric fluid component, and for miscellaneous minor uses. They are also found as contaminants of trichlorobenzenes and, therefore, there is potential exposure to tetrachlorobenzenes where exposure to the former occurs. Thus, occupational exposure during manufacturing, processing, and use is likely. Human exposure via the food chain is also a possibility. Exposure potential is increased because the physicochemical properties of tetrachlorobenzenes indicate a potential for persistence in soil and water systems and for bioaccumulation.

E. Pentachlorobenzene

Pentachlorobenzene, also known as quintochlorobenzene, is a crystalline solid. It is moderately soluble in benzene, carbon tetrachloride, chloroform, ether, and carbon disulfide but insoluble in cold alcohol. Its water solubility is expected to be lower than those of tri- and tetrachlorobenzenes (compare trend of data in Table 1, Section I). Pentachlorobenzene can be produced by the catalyzed chlorination of any of the tetrachlorobenzenes. It may also be formed in small amounts when trichloroethylene is heated to 700°C (Hardie 1964).

The TSCA Inventory lists one manufacturer of pentachlorobenzene for 1977, with production volume reported in the one to ten million pounds per year range (OPTS 1979b). Pentachlorobenzene is nitrated to produce the soil fungicide and seed disinfectant pentachloronitrobenzene (PCNB), also called Quintozene^R (Thomson 1975); Dow (1979) states that all pentachlorobenzene produced intentionally (i.e., not as a by-product) is converted to PCNB at the site of manufacture. The final product contains about 0.2 percent pentachlorobenzene (Beck and Hansen 1974). According to Dow, the pentachlorobenzene that is formed during the manufacture of other chlorinated benzenes amounts to less than one million pounds per year and is all disposed of as waste (Dow 1979); the method of disposal was not discussed. However, pentachlorobenzene was shown to comprise 2.5% by weight of a transformer fluid (Lewis 1979), a use of chlorinated benzenes that may be increasing because of controls that are being placed on PCB's.

Pentachlorobenzene has been identified in the tissues of fish from the Saginaw River (Mich.), Ashtabula River (Ohio), Wabash River (Ind.), Arkansas River (Ark.), Mississippi River (La., Tenn.), White Lake and Tittabawassee River (Mich.), Niagara River (N.Y.) and Lake Ontario (Veith et al. 1979, Yurawecz 1980). It has been detected in Great Lakes herring gull eggs at concentrations up to 30 ppb (International Joint Commission 1979) and in Great Lakes coho salmon at unspecified levels (International Joint Commission 1978).

Pentachlorobenzene has been found at levels of 0.001 to 0.006 ppm in oils, fats and shortening, and has been detected in sugar at a concentration of about 0.002 ppm (Johnson and Manske 1977). Recently a level of 0.11 ppm in peanut oil has been reported (Lombardo 1979). The occurrence of pentachlorobenzene in agricultural products may be a result of pesticide uses.

Pentachlorobenzene is persistent and may remain in soil for up to two or three years after application (Beck and Hansen 1974).

Conclusion: Pentachlorobenzene is manufactured in the one to ten million pounds/year range for use as a chemical intermediate. It also is a contaminant of lower chlorobenzenes and a by-product of their manufacture and, therefore, may have similar exposure patterns; its occurrence in a transformer fluid containing tri- and tetrachlorobenzenes is an example. The known soil persistence of pentachlorobenzene, its occurrence in natural waters and in fish and bird tissues, and its physicochemical properties all point to a potential for persistence and bioaccumulation that magnifies the above-noted exposure concern.

III. Health Effects

A. Acute Effects

1. Evaluation of Pertinent Studies

a. Human Case Reports

Most reported incidents of human toxicity due to chlorinated benzenes resulted from inhalation exposure. Accidental inhalation can occur either in the home or at the workplace. There are also incidents in which accidental or deliberate ingestion of chlorinated benzenes has occurred.

Monochlorobenzene is a central nervous system depressant and will cause symptoms typical of its anesthetic effect. Exposure to monochlorobenzene causes headaches, irritation of the eyes and upper respiratory tract, numbness, and eventual loss of consciousness (Irish 1963). Ehrlicher (1968) described a case in which a worker inhaled massive amounts of monochlorobenzene while repairing a pump which was spewing a thick spray of the chemical. After a few hours he experienced massive hemoptysis (coughing up of blood); examination with X-rays did not identify an alternative cause for the reaction.

Sensitization to o-dichlorobenzene was reported by Downing (1939) for a man who regularly handled window sashes dipped in a mixture containing the compound. Subsequently when o-dichlorobenzene was applied to the skin of this individual there was a positive reaction within 2 minutes. Intense erythema and edema developed at the site of application and for one-half inch surrounding it. Later a large bullous lesion formed in the center of this area.

An industrial hygiene survey (Dow 1978c) noted that, in the production of p-dichlorobenzene, "at least some of the operators had a dermatitis on the inside of the forearms, apparently from their exposure during the pulling of the cakes." They reported further that p-dichlorobenzene vapor at concentrations of 1-3 mg/liter (17-500 ppm) produced "severe eye irritation, even in those men who are accustomed to the vapor." Although these observations do not prove that this compound caused dermal sensitization, they appear to rule out any lessening of sensitivity through habituation.

Odor-threshold data have been reported on monochlorobenzene and o- and p-dichlorobenzenes (Varshavskaya 1967, Tarkhova 1965). Although odor is not usually considered to be a health hazard, it may be useful to consider olfactory data. Odor signals the fact of exposure and of increasing ambient concentrations and thus may presage the development of adverse health effects as the concentration in air increases. It may be useful to know the margin between the olfactory threshold and the concentration(s) at which adverse health effects occur. Odor triggers more complaints from the general public than do some of the more significant adverse health effects. Vague health complaints, such as disturbances of sleep or digestion, are frequently associated with odor nuisances.

No-effect levels for eye and respiratory irritation in humans have also been reported for o-dichlorobenzene and p-dichlorobenzene. Hollingsworth et al. (1958) reported that neither eye nor respiratory irritation was evident in workers exposed to o-dichlorobenzene at concentrations of 1-44 ppm (6-265 mg/m^3) with an average concentration of 15 ppm (90 mg/m^3). Exposure at 100 ppm (600 mg/m^3) had produced such irritation (Elkins 1950).

Workers exposed to p-dichlorobenzene at concentrations of 15-88 ppm (90-510 mg/m^3), with an average of 45 ppm (270 mg/m^3), did not consider these levels to be objectionable, although they had complained of eye and nasal irritation at 50-170 ppm (300-1,020 mg/m^3) with an average exposure of 105 ppm (630 mg/m^3) (Hollingsworth et al. 1956).

Mussell et al. (1958) asserted that 1,2,4,5-tetrachlorobenzene might be slightly irritating to the eyes or skin if direct contact with the eyes or prolonged or repeated contact with the skin occurred. Supporting data and quantitative levels were not presented.

There may be two explanations, not mutually exclusive, for the different responses seen with similar exposure conditions as, for example, in the concentration ranges of p-dichlorobenzene reported by Hollingsworth et al. (1956) to be irritating or non-irritating. One explanation is that individual differences in sensitivity or susceptibility are most evident at threshold concentrations. The other explanation may reside in differences in the duration of exposure to a given concentration, as suggested by Mussell et al. (1958), with longer exposure producing greater irritation.

Dupont (1938) described the experiences of French sewage workers exposed to the effluents of a dry cleaning establishment using o-dichlorobenzene. Vapors emanating from the sewer floor caused lacrimation and vomiting, and the workers experienced a distaste for cigarette smoking.

There is more experience with human exposure to p-dichlorobenzene than to other chlorobenzenes, due to its widespread use as a moth repellent and deodorizer. Exposed individuals have exhibited various responses including weakness, headache, rhinitis, twitching of facial muscles, and acute hemolytic anemia with methemoglobinuria (Cotter 1953, Hallowell 1959).

One case of acute p-dichlorobenzene poisoning was reported by Cotter (1953). It involved a 35-year old housewife who, following use of a commercial moth killer, experienced periorbital swelling, intense headache, and profuse rhinitis; all symptoms subsided in 24 hours.

The case of a 3-year-old boy who presumably ingested "Demothing Crystals" containing p-dichlorobenzene was reported by Hallowell (1959). The child developed acute hemolytic anemia with methemoglobinuria. Analysis of his urine, which was markedly reduced in volume during the first 2 days after poisoning, revealed traces of 2,5-dichloroquinol, but no 2,5-dichlorophenol. Examination approximately one month after the incident indicated an apparently complete recovery.

b. Animal Studies

Animal toxicity determinations have been conducted for monochlorobenzene, o-dichlorobenzene, p-dichlorobenzene, and 1,2,4-trichlorobenzene in various animal species including mice, rats, rabbits, and guinea pigs. The LD_{50} values determined in these studies are presented in Table 1.

Additional studies include reports of mortality following short term exposure to chlorobenzenes. Irish (1963) cited the results of an acute study of monochlorobenzene vapor in cats. The cats were exposed presemably continuously, to monochlorobenzene vapor at concentrations of 220 to 8,000 ppm. Concentrations of 220-660 ppm were tolerated well for an hour; definite narcotic signs were noted at 1,200 ppm; unsteadiness, tremors, and twitching occurred at concentrations of 2,400 to 2,900 ppm. Death occurred after 7 hours in animals exposed to 3,700 ppm. At the highest concentration, 8,000 ppm, severe narcosis occurred after one-half hour, and the cats died two hours after removal from exposure.

The Eastman Kodak Company (1978) reported that acute inhalation of 22,000 ppm of monochlorobenzene killed two-thirds of the rats tested in 2.5 hours while inhalation of 9,000 ppm of monochlorobenzene killed two-thirds of the rats in 3 hours.

Hollingsworth et al. (1958) exposed male rats via inhalation to one of four different concentrations of o-dichlorobenzene for 1 to 10 hours. The results are presented in Table 2. The average vapor concentration ranged from approximately 500 to 1,000 ppm and the numbers of animals ranged from 5 to 20 per group. During the exposure period, the rats exhibited drowsiness, unsteadiness, eye irritation, difficulty in breathing, and anesthesia. The intensity of visible responses was dependent on the concentration and the duration of exposure. Most of the deaths occurred within three days after removal of the animals

TABLE 1: ACUTE TOXICITY DETERMINATIONS

Compound	Route	Species	LD_{50} (mg/kg unless specified)	Reference
Monochlorobenzene	Oral	Rat	2144	Monsanto 1965c
	Oral	Rat	400-1600	Eastman Kodak 1978
	Oral	Rabbit	2830	Eastman Kodak 1978
	Dermal	Rabbit	>10 g/kg	Monsanto 1965c
o-Dichlorobenzene	Oral	Rat	1516	Monsanto 1965a
	Dermal	Rabbit	>10 g/kg	Monsanto 1965a
m-Dichlorobenzene	Oral	Rat	400-3200	Eastman Kodak 1978
p-Dichlorobenzene	Oral	Mouse	2950	Domenjoz 1946
	S.C.	Mouse	5145	Irie et al. 1973
	Oral	Rat	2620	Monsanto 1959
	Oral	Rat	1625	Monsanto 1965b
	Oral	Rat	1860	Monsanto 1975
	I.P.	Rat	2562	Zupko and Edwards 1949
	Dermal	Rabbit	>21.4 g/kg	Monsanto 1965b
	Dermal	Rabbit	>5.01 g/kg	Monsanto 1975
	Oral	Guinea pig	2800	Eastman Kodak 1978
1,2,4-Trichlorobenzene	Oral	Mouse	766	Brown et al. 1969
	Oral	Rat	756	Brown et al. 1969
	Percut.	Rat	6139	Brown et al. 1969
Pentachlorobenzene	Oral	Mouse	1175-1370	Linder et al. 1969
	Oral	Rat	940-1125	Linder et al. 1969

from the chamber. The same investigators also exposed matched groups of 20 male rats to 977 ppm of *o*-dichlorobenzene for 1 hour and 0.5 hour and to 539 ppm for 6.5 hours and 3 hours. Central lobular necrosis in the liver and cloudy swelling of the kidney tubular epithelium were observed in all groups except those exposed to 997 ppm for 0.5 hour. The degree of damage was greater in the case of the longer exposures.

TABLE 2

Mortality of Male Rats Exposed to Various Concentrations of *o*-Dichlorobenzene Vapor for Single Periods (Hollingsworth et al. 1958)

Average Vapor Concentration By Analysis (ppm)	Period of Exposure (hr)	No. of Rats Exposed	Number of Deaths
977	10	5	5
	7	5	4
	2	20	0
	1	5	0
941	7	5	2
	4	20	1
	2	5	1
	1	5	0
821	7	20	5
539	7	5	0

Hollingsworth et al. (1958) also fed *o*-dichlorobenzene to guinea pigs by intubation as a 50 percent solution in olive oil. All of 10 animals receiving a single dose of 0.8 g/kg survived, while a dose of 2.0 g/kg produced 100 percent mortality in 10 guinea pigs.

Cameron et al. (1939) studied the acute toxicity of *o*-dichlorobenzene in rabbits and mice. Intravenous injection of 0.25 to 0.5 ml/kg was fatal to rabbits within 24 hours. In mice the minimal lethal dose following injection was found to be about 0.4 ml/kg. Mice, rats, and guinea pigs were exposed to air containing 0.005 to 0.080 percent *o*-dichlorobenzene from 30 minutes to 50 hours. Under these conditions, the animals exhibited reduced activity, drowsiness, and irritation of eyes, followed by death. Autopsy revealed liver necrosis in nearly all animials.

Varshavskaya (1967) studied acute exposure to monochlorobenzene, o-dichlorobenzene, and p-dichlorobenzene in mice, rats, rabbits, and guinea pigs. The adverse effects included asthenia, inactivity, loss of weight and appetite, and distinct narcotic effects followed by death within three days due to paralysis of the respiratory center.

Results of an acute toxicity study in rats and mice on pentachlorobenzene carried out at EPA's Health Effects Research Laboratory have been submitted for publication and will be included in this document when the prepublication review is complete. The LD_{50}'s for this compound are around 1 g/kg, consistent with other chlorobenzenes tested.

2. Decision

The two major acute effects of the chlorinated benzenes are tissue irritation and central nervous system depression. Eye, skin, and respiratory irritation, rhinitis, and periorbital swelling have been associated with exposure to the lower chlorinated benzenes (monochlorobenzene, o- and p-dichlorobenzene). Skin sensitization may also occur. Central nervous system depression leading to loss of consciousness, headache, and vomiting have been reported following acute human exposures, which generally occur by accidental inhalation. Although high concentrations of chlorinated benzenes may be lethal to laboratory animals, human exposures of comparable magnitude are unlikely. Threshold limit values in the air of the work place adopted for several chlorinated benzenes by the U.S. Occupational Safety and Health Administration are given in Appendix C of this support document.

In general, the acute toxicity of the chlorinated benzenes has been adequately characterized. The available LD_{50} data are on the order of the 1000 mg/kg or higher, and there is no reason to expect significant deviations from this range for the untested category members. Two reports (Downing 1939, Dow 1978c) indicated that prolonged, heavy exposure to o- or p-dichlorobenzene may induce dermal sensitization; however, the Agency is not aware of any indication that there is a serious or widespread problem posed by dermal exposure to the chlorinated benzenes. The Agency is, therefore, not requiring testing for dermal sensitization at this time. Although a more rigorous and systematic determination of the thresholds for acute skin, eye, and mucous membrane irritation and for acute central nervous system depression could be undertaken for all of the chlorinated benzenes, such testing is not needed at this time for regulatory purposes and is not being proposed.

B. Subchronic and Chronic Effects

1. Evaluation of Pertinent Studies

Note: This part excludes effects on the central nervous system, which are discussed under Neurotoxic Effects, part C of this Section.

a. Human Case Reports

The case histories presented here provide only suggestive evidence because the absence of controlled conditions makes it difficult to determine cause and effect.

A number of incidents of systemic toxicity in humans exposed to chlorinated benzenes have been reported. Toxic effects on the liver have been reported by several authors (Berliner 1939, Sumers et al. 1952, Cotter 1953). The report by Cotter presents an example of the type of liver toxicity generally noted. He described several cases of liver damage associated with exposure to p-dichlorobenzene. The first case involved a woman who demonstrated products containing p-dichlorobenzene and who complained of tiredness, nausea, headache, and vomiting. Her conjunctivae were muddy with a yellow tinge, and she had elevated serum bilirubin. An x-ray of the esophagus showed varices. The final diagnosis of her case was subacute yellow atrophy and cirrhosis of the liver. The second case involved the death of a husband and wife whose house had been saturated with mothball vapor for a period of at least three to four months. Autopsy confirmed the cause of death as acute yellow atrophy of the liver for both patients and Laennec's cirrhosis and splenomegaly for the wife. Both had elevated serum alkaline phosphatase (SAP) and serum bilirubin. Cotter also reported the case of a 52-year-old trapper who had used p-dichlorobenzene for two years in the curing of raw furs. The subject showed a consistent picture of subacute yellow atrophy of the liver, accompanied by jaundice, elevated serum bilirubin and SAP, and a palpable spleen. All of the above cases displayed a picture of yellow atrophy with a tendency toward connective tissue formation rather than liver cell regeneration. The husband and wife showed anemia and borderline anemia, respectively.

Toxic effects on the hematopoietic system and the formed blood elements due to exposure to chlorinated benzenes have also been observed by several authors (Perrin 1941, Petit and Champeix 1948, Wallgren 1953, Gadrat et al. 1962, Campbell and Davidson 1970, Girard et al. 1969). The following three reports indicate the variety of effects noted .

Wallgren (1953) reported the case of eight men who worked from one to seven months in a factory manufacturing mothproofing agents produced from p-dichlorobenzene that contained approximately one percent of an unidentified nitrogen-containing impurity and a small amount of o-dichlorobenzene. Blood examinations showed methemoglobinemia and varying degrees of lymphocytosis in all cases, thrombocytopenia in four cases, and leukopenia and peripheral granulocytopenia in one case.

Campbell and Davidson (1970) reported the unusual case history of a pregnant woman who developed a pica for p-dichlorobenzene. Each week throughout her pregnancy, the 21-year-old woman consumed one to two toilet air-freshener blocks composed primarily of p-dichlorobenzene. She was diagnosed as having hypochromic anemia and toxic hemolytic anemia with Heinz body formation. When she stopped eating the chemical, the woman made an apparently complete recovery. In blood tests six weeks after delivery, hemoglobin levels, erythrocyte morphology, and reticulocyte numbers were normal. Neonatal examination of the child revealed no apparent abnormalities.

Girard et al. (1969) reported severe aplastic anemia in a 68-year-old woman exposed to trichlorobenzene. Although the exact composition of the fluid to which she was exposed was unknown, it was believed by the authors to be primarily trichlorobenzene. Following the woman's hospitalization, an examination revealed severe aplastic anemia. When the patient died one month later, she was hemorrhaging. The autopsy results indicated a retroperitoneal hematoma.

b. Animal Studies

Summaries of the available animal studies are given in Tables 3-5 at the end of this Section b. Only those reports most significant for hazard assessment are presented here.

(1) Monochlorobenzene

(a) Oral Studies (capsule or gavage)

A 13-week oral toxicity study was reported by Monsanto Co. (1967a). Thirty-two beagles, 8 animals per group (4 males, 4 females), were administered 0, 0.025, 0.050, and 0.250 ml/kg/day (27.3 mg/kg/day, 54.6 mg/kg/day, and 272.5 mg/kg/day) of monochlorobenzene orally by capsule 5 days per week for 13 weeks. At the highest dose, two of the 8 dogs died, and two other dogs were sacrificed in moribund condition between the third and fifth weeks of the study. The surviving animals were sacrificed after 13 weeks of administration. All of the dogs in the high dose group showed weight loss: 3.1 kg for each of the two dogs that died; 4.2 kg for each of the two dogs sacrificed; and 0.7-2.0 kg for the four dogs surviving to termination of the study. Clinical studies conducted on three of the four dogs that died or were sacrificed early revealed the following: increased count of juvenile and band cells (marked in two dogs); increased count of monocytes (marked in one dog); sharply decreased lymphocyte count; low blood sugar levels; elevated serum glutamicpyruvic transaminase (SGPT), serum alkaline phosphatase (SAP), and total bilirubin (bilirubin in the urine of two of the dogs was significantly elevated also); and elevated total cholesterol in two of the dogs. The results of hematological studies and urine analyses for the four animals surviving to termination were at one and three months comparable to corresponding initial values. Biochemical studies on these latter animals showed a slight increase in SGPT values for the two male dogs and a slight increase in SAP values for one male and one female. Histologic changes were observed in the liver (centrolobular fibrosis), kidney, gastrointestinal mucosa, and hematopoietic tissue in all eight dogs in the high dose group. Slight and somewhat inconsistent histologic changes were observed in the livers of the dogs at the intermediate dose. No reported toxic effects were observed in the dogs at the low dose.

Monsanto (1967b) reported the results of a 90-day oral study on monochlorobenzene using albino rats. Repeated doses of 12.5 mg/kg/day for 90 days produced an increase in salivation for 5 to 10 minutes in 1/36 rats (18 males, 18 females tested) and alopecia in 1 rat. No clinical signs or histopathological alterations were noted. Doses of 50.0 mg/kg/day for 90 days produced an increase in salivation in 18/36 rats, and 1/36 animals died just prior to sacrifice. No compound-related clinical changes or histopathological alterations were observed. Doses of 100 mg/kg/day produced increased salivation in the majority of animals and alopecia in 1/36 rats. One animal died on day 88. No clinical signs of histopathological alterations were observed. Doses of 250/mg/kg/day produced increased salivation in all of the animals, alopecia in 4/36 animals, and a significant decrease in growth rate for males. One animal died on day 90. No clinical signs or histopathological lesions were observed. In this study no vehicle controls were used (only negative controls) and histopathology was performed only on 5 animals of each sex per dose group. Extensive microscopic studies were done on the highest dose group and on controls and only the thyroid, heart, kidney, liver, and adrenals were examined in the low and intermediate dose groups.

Varshavskaya (1967) administered 0, 0.1, 0.01, and 0.001 mg/kg of monochlorobenzene in sunflower oil by stomach tube daily for nine months to male albino rats (7 animals/dose level). At the highest dose the compound caused a statistically significant inhibition of erythropoiesis, thrombocytosis, and an inhibition of mitotic activity in the marrow. Eosinophilia was also noted as well as an increase in the phagocytic activity of leukocytes, SAP, serum transaminase activity, and the gamma-globulin plasma protein fractions. The intermediate dose produced all the effects noted above except the phagocytic activity of leukocytes. These effects occurred to a lesser degree, but it was not reported whether they were statistically significant. No effects were noted at the lowest dose level. No indication was given whether the term "daily" meant 7 days/week or 5 days/week.

(b) Inhalation Studies

The Monsanto Co. (1978g) reported the results of a 90-day inhalation toxicity study on monochlorobenzene using beagle dogs and Charles River albino rats. Prolonged inhalation of 0.75 mg/l air, 6 hours per day, 5 days per week for a total of 62 exposures (12.4 weeks) did not cause any adverse effects in dogs (4 males, 4 females). However, only urinalysis, clinical chemistry, and blood studies were done at this dose and no histopathological examinations were performed. Prolonged inhalation of 1.50 mg/l air (325 ppm) for the same time period caused a decrease in weight, conjunctivitis in 2/8 dogs, and hypoactivity in 2/8 dogs. The dogs were sacrificed in moribund condition before the thirty-first day. Gross examination revealed evidence of icterus in 1 of these 2 dogs. No histopathological examination was performed in any of the dogs at this dose level and no clinical chemistry, urinalysis or blood studies were done on the 2 dogs sacrificed prior to termination of the experiment. Prolonged inhalation of 2.00 mg/l air (434 ppm) for the same time period caused a significant decrease in weight, hypoactivity, and conjunctivitis in 8/8 dogs (4 males, 4 females). Mean values for total leukocyte counts after 45 and 90 days of treatment were lower than corresponding controls and mean values for SAP, serum glutamate oxaloacetate transaminase (SGOT), and SGPT were elevated after 38 days of treatment. Histopathologic examination revealed vacuolation of hepatocytes and aplastic bone marrow in 5 dogs, hypoplastic bone marrow in 1 dog, cytoplasmic vacuolation of the epithelium of the renal collecting tubules in 4 dogs, and bilateral atrophy of the seminiferous epithelium in the testes of 2 dogs. Five dogs were sacrificed in moribund condition between the twenty-fifth and thirty-eighth day of the experiment.

Prolonged inhalation of 0.75, 1.50, or 2.00 mg/l of monochlorobenzene by rats for the same time period as for the dogs revealed erythemia and alopecia in 2/30 animals in the lowest dose group and variable organ weights in all dose groups (15 males, 15 females per group). Clinical chemistry, urinalysis, and blood chemistry were done on only 5 males and 5 females from the highest dose group and from the controls; the values determined for these parameters fell within the normal range. Histopathology was performed on controls and on the highest dose group (all animals in these groups); no tissue changes attributable to the effects of monochlorobenzene were observed.

Dilley (1977) exposed 3 groups of 32 male rats and rabbits to 0, 75 and 250 ppm of monochlorobenzene for 7 hours/day, 5 days/week, for up to 120 exposure days (24 weeks). Clinical changes included hematological changes in both treated rat groups at 11 and 24 weeks (changes at 11 weeks were significant), a decrease in SGOT activity in high dose rats, and minor hematological changes in both groups of rabbits at 11 and 24 weeks. No significant histopathologic changes were observed in the rabbits or rats at 24 weeks.

Khanin (1977) exposed rats to 0.1 and 1.0 ug/l (0.022 ppm and 0.22 ppm) of monochlorobenzene 24 hours/day for 70-82 days. Three to five rats were used in each group and corresponding controls were tested. Toxic encephalopathy, granular albumin, dystrophy of the heart and liver, non-acute nephrosis of the kidneys with limited glomerulonephritis, hyperplasia of the follicles of the spleen, and proliferation of reticular cells of the arterial sheaths were noted. Inflammation of the heart and liver were also observed. The effects were greater with the higher dose.

Other monochlorobenzene animal studies are summarized in Table 3 at the end of this Section b.

(2) Dichlorobenzenes

(a) Oral Studies

Groups of 10 young adult female white rats were given doses of 18.8, 188, or 376 mg/kg of p-dichlorobenzene (10 controls) in olive oil emulsified with about 2 ml of 5 to 10 percent acacia (gum arabic) (Hollingsworth et al. 1956), or o-dichlorobenzene in olive oil (20 controls) (Hollingsworth et al. 1958) by stomach tube 5 days per week for a total of 138 doses in 192 days. Animals were sacrificed one day after the final dose. At the highest dose level both compounds were associated with a moderate increase in liver weight; p-dichlorobenzene was associated with slight cirrhosis and focal necrosis of the liver; and o-dichlorobenzene was associated with slight to moderate swelling of the liver. For both compounds only slight increases in liver and kidney weights were observed with the intermediate dose level; the lowest level produced no changes.

A group of five rabbits of both sexes were administered a 25.0 percent solution of p-dichlorobenzene in olive oil (intubation) 5 days a week for a total of 92 doses in 219 days (Hollingsworth et al. 1956). The dosage level was 1000 mg/kg/day. Another group of seven rabbits received the compound 5 days a week for a total of 263 doses in 367 days at a level of 500 mg/kg/day. Slight changes in the liver characterized by cloudy swelling and a few areas of focal, caseous necrosis were found at both dose levels.

Varshavskaya (1967) reported the same type of studies for o-dichlorobenzene that were done for monochlorobenzene. Increases in urinary 17-ketosteroids, in the phagocytic activity of leukocytes (at 0.1 mg/kg/day), and in the gamma-globulin plasma protein fractions (at 0.01 and 0.1 mg/kg/day) were found, as well as a statistically significant inhibition of erythropoiesis, thrombocytosis, and inhabition of mitotic activity in the bone marrow (at 0.1 mg/kg/day), when the chemical was administered orally to rats for a period of 9 months. The same effects were produced with 0.01 mg/kg/day but to a lesser degree. No effects were reported at 0.001 mg/kg/day.

(b) Inhalation Studies

Hollingsworth et al. (1956) exposed various sized groups of rats, rabbits, guinea pigs, and other species to p-dichlorobenzene vapors at each of five concentrations for 7 hours/day (8 hours/day for the highest dose group), 5 days/week. In the rat, 19 males and 15 females were exposed to 4.8 mg/l (798 ppm) for up to 69 exposures. Cloudy swelling and central necrosis of the liver were observed in both sexes, and slight cloudy swelling of the tubular epithelium of the kidneys was observed in the females. Increased liver and kidney weights were observed at concentrations of 2.05 mg/l (341 ppm: 20 male rats tested for 6 months) and 1.04 mg/l (173 ppm: 5 male, 5 female rats tested for 16 days). At 0.95 mg/l (158 ppm), 20 rats (10 males, 10 females) were given 139 exposures over 199 days. Increased liver weights and centrolobular hepatocellular cloudy swelling or granular degeneration of questionable significance in the parenchymal cells in the central zones were noted. No adverse effects were observed in 20 rats (equal number of males and females) exposed to 0.58 mg/l (96 ppm) for six to seven months.

Liver toxicity was observed in guinea pigs exposed to similar concentrations as the rats. Cloudy swelling and central necrosis of the liver were seen at 798 ppm (16 males, 7 females tested for up to 23 exposures); vacuolization, fatty degeneration, focal necrosis, and slight cirrhosis were seen in some males at 341 ppm for 6 months (8 males, 8 females tested). Decreased spleen weights in males were reported at 173 ppm (exposed for 16 days), increased liver weights in females were observed at 158 ppm (exposure period was 157-219 days), and no adverse effects were noted at 96 ppm.

The picture was similar for rabbits. Cloudy swelling and central necrosis of the liver were reported at 798 ppm and some lung effects at 173 ppm. No other adverse effects were noted at any of the other dose levels. No effects were reported for mice and a monkey exposed to either 158 ppm from 157 to 219 days or 96 ppm for 6 to 7 months.

There appeared to be no rationale for the choice of dose levels, number of animals, and species. However, the results of the study indicated systemic effects in several species.

Hollingsworth et al. (1958) also exposed animals by inhalation to o-dichlorobenzene. Groups of 20 rats, 8 guinea pigs, and 2 rabbits of each sex and 2 female monkeys were exposed to 0.56 mg/l (93 ppm) 7 hrs./day, 5 days/week for periods ranging from 6 to 7 months. The only toxic effect noted was a statistically significant decrease in the average spleen weight of male guinea pigs. When groups of 20 rats and 8 guinea pigs of each sex and 10 female mice received 0.29 mg/l (49 ppm) of o-dichlorobenzene 7 hrs./day, 5 days/week for six and one half months, no gross or microscopic abnormalities were observed.

Imperial Chemical Industries in Great Britain is reported to be carrying out a long-term inhalation study in rats on p-dichlorobenzene (Dow 1978b). EPA is trying to learn more about this study, and the Agency will include any available data in its ongoing evaluation of the chlorinated benzenes.

Animal studies involving dichlorobenzenes are summarized in Table 4 at the end of this Section b.

(3) Trichlorobenzenes

Coate et al. (1977) exposed 4 groups of 30 young male Sprague-Dawley rats, 4 groups of 16 male albino rabbits, and 4 groups of 9 male cynomolgus monkeys to 0, 0.183, 0.37, or 0.73 mg/l (0, 25, 50, or 100 ppm) of 1,2,4-trichlorobenzene (99.07 percent pure) for 26 weeks, 7 hours per day, 5 days per week.

The microscopic change observed was hepatocytomegaly in rats after 4 weeks of exposure. The changes were greater in animals exposed to 492 and 95.8 ppm than in those exposed to 25 ppm. Hyaline degeneration was present in the inner zone of the kidney cortex in all test groups after 4 weeks and 13 weeks of exposure. These changes were transient, however, and no exposure-related abnormalities were seen after 26 weeks. A no-effect dose was not established. No toxic effects were observed in the other species at any dose level.

(4) Tetrachlorobenzenes

Fomenko (1965) reported toxic effects on the liver and hematopoietic system in rabbits and rats following administration of 1,2,4,5-tetrachlorobenzene. Rats were given 75 mg/kg by gavage daily for two months. Decreased liver function was reported. The prothrombin index dropped by almost one-third in comparison to the control group. There was a statistically significant increase ($p= 0.01$) in the activity of the blood cholinesterase (author did not state whether pseudo- or acetylcholinsterase). The number of reticulocytes in the peripheral blood first decreased ($p= 0.02$) and then increased; at the end of the experiment, the toxic signs included erythemia ($p= 0.01$), an increased number of large-diameter erythrocytes, and some decrease in the serum potassium. Microscopic examination revealed no tissue abnormalities. The same tetrachlorobenzene isomer was administered in vegetable oil by gastric intubation to rabbits at 0, 0.05, 0.005, and 0.001 mg/kg daily for eight months. Increased retention of an intravenous load of galactose in the blood began at 6 months in the group receiving 0.05 mg/kg, suggesting to the author interference with the glycogen-forming function of the liver. A significant ($p= 0.01$) reticulocytosis was noted by the end of the eighth month. In rabbits given 0.005 mg/kg, a brief disorder of glycogen formation was reported in the eighth month of the study. No effects were observed at the lowest dose level. The number of animals tested was not given in the report.

Trichloro- and tetrachlorobenzene animal studies are summarized in Table 5 at the end of this Section b.

(5) Pentachlorobenzene

A study of subchronic toxicity of pentachlorobenzene in rats performed at EPA's Health Effects Research Laboratory, Research Triangle Park, NC., has recently been submitted for publication (Linder et. al., 1980). Initial evaluation of the study by EPA indicates that the toxicity of pentachlorobenzene is similar to that of other chlorinated benzenes: the liver, kidney and nervous system are the primary targets. Provided that editorial review of the study raises no major questions, EPA considers this study to characterize adequately the subchronic toxicity of pentachlorobenzene for risk assessment purposes.

TABLE 3--MONOCHLOROBENZENE
ORAL* SUBCHRONIC TOXICITY STUDIES

Compound	Species	Dose and Duration	Effect	Ref.
Monochlorobenzene	Rats	0.1 mg/kg/day for 9 mo.	Statistically significant inhibition of erythropoiesis, thrombocytosis, and mitotic activity in marrow; inhibited higher cerebral cortex activity, eosinophilia.	Varshavskaya 1967
Monochlorobenzene	Dogs	0.050 ml/kg/day for 13 weeks	Diarrhea, vomiting, conjunctivitis.	Monsanto 1967a
Monochlorobenzene	Dogs	0.250 ml/kg/day for 13 weeks (272.5 mg/kg/day for 93 days, 5 days/week)	Cachexia; icterus; hepatic and renal discoloration; distended gall bladder; increased organ/body weight ratios for internal organs; death; increased monocytes; decreased number of leukocytes. Increased number of immature leukocytes, low blood sugar, elevated SGPT and alkaline phosphatase, increased total bilirubin and total cholesterol, gross and/or microscopic pathology of hematopoietic, liver, and kidney tissues.	Monsanto 1967a
Monochlorobenzene	Rats	12.5, 50, 100, and 250.0 mg/kg/day for 93 to 99 days	Retarded growth of male rats (high level (sic)). Liver and kidney weights significantly increased (100 and 250 mg levels).	Monsanto 1967b

Administered by gavage or capsule.
The doses reported are the lowest effective dose for each study.

TABLE 3--MONOCHLOROBENZENE (cont.)
SUBCHRONIC INHALATION TOXICITY STUDIES

Compound	Species	Dose and Duration	Effect	Ref.
Monochlorobenzene	Rats	0.1 or 1.0 mg/m^3 for 70-82 days, continuous	Encephalopathy, interstitial pneumonia, inflammation of internal organs, protein dystrophy in liver, regenerated cells in liver, focal giant-cell hyperplasia in kidneys.	Khanin 1969
Monochlorobenzene	Rats	1.0 mg/m^3, continuously for 60 days	Distorted chronaxia of antagonist muscles, increased blood cholinesterase activity, lowered alpha-globulin and increased beta-globulin content of blood serum.	Tarkhova 1965
Monochlorobenzene	Rats	0.1 or 1.0 mg/1, 7-14 weeks	Chronaximetric inhibition preceding enzymatic changes.	Pislaru 1960
Monochlorobenzene	Rats	0.1 mg/l for 3 hrs., alternate days for 37 weeks	Temporary inhibition of extensor tibialis chronaxia after 7-14 weeks.	Gabor, Raucher 1960
Monochlorobenzene	Dogs	2.00 mg/1, 6 hrs./day, 5 days/week, 62 exposures	Enlarged and hardened liver, vacuolated hepatocytes, cytoplasmic vacuolation of renal collecting tubules, bilateral atrophy of seminiferous tubules, lower total leukocyte counts, elevated SAP, SGOT, SGPT, aplastic bone marrow, 5/8 dogs died after 25-29 days.	Monsanto 1978g
Monochlorobenzene	Rats and Rabbits	75 or 250 ppm, 7 hrs./day, 5 days/week, up to 120 exposure days	Suggestion of microcytic anemia, decrease in SGOT activity, focal lesions of adrenal cortex, tubular lesions in kidney, congestion in liver and kidneys in rats only. Signigicant changes in clinical chemistry profile in both species.	Dilley 1977
Monochlorobenzene	Rats Guinea Pigs	200 ppm, 6hr/day, 10 weeks	(No effects seen)	Eastman Kodak

TABLE 3--MONOCHLOROBENZENE (cont.)
SUBCHRONIC AND CHRONIC DIETARY TOXICITY STUDIES

Compound	Species	Dose and Duration	Effect	Ref.
Monochlorobenzene	Rats	1140 mg/kg/day for 5 days	Increased liver and urinary coproporphyrin, uroporphyrin, porphobilinogen, delta-aminolevulinic acid; fatty and necrotic liver damage.	Rimington, Ziegler 1963

SUBCHRONIC PARENTERAL TOXICITY STUDIES

Compound	Species	Dose and Duration	Effect	Ref.
Monochlorobenzene (technical product containing up to 30% benzene and 1.1% dichlorobenzene)	Rabbits	0.9 mg/kg/injection for up to 20 injections	Swelling of convoluted tubule epithelium and glomeruli in kidneys, cellular swelling and fat deposition in liver.	Rozenbaum 1947

TABLE 4--DICHLOROBENZENES
ORAL* SUBCHRONIC TOXICITY STUDIES

Compound	Species	Dose and Duration	Effect	Ref.
1,2-Dichlorobenzene	Rats	376 mg/kg, 138 doses in 192 days	Increased liver and kidney weight, decreased spleen weight, cloudy liver swelling.	Hollingsworth 1958
1,2-Dichlorobenzene	Rats	0.1 mg/kg/day for 9 mo.	Statistically significant inhibition of erythropoiesis, thrombocytosis, and mitotic activity in marrow, inhibited higher nervous activity in cerebral cortex, neutropenia.	Varshavskaya 1967
1,4-Dichlorobenzene	Rats	376 mg/kg/day, 138 doses	Increased weight of liver and kidney, decreased weight of spleen, liver cirrhosis and focal necrosis.	Hollingsworth 1956
1,4-Dichlorobenzene	Rabbit	500 mg/kg, 263 doses in 367 days; 1000 mg/kg, 5 days per week, 92 doses in 219 days.	Swelling and focal necrosis of liver; higher doses caused some deaths.	Hollingsworth 1956
1,4-Dichlorobenzene	Rabbit	1 g/kg, 5 days/week (duration not specified)	Intoxication, weakness, tremors, weight loss, death.	Pike 1944
1,3-Dichlorobenzene	Rat	800 or 900-1000 mg/kg/day for 1 to 5 days	Hepatic porphyria (900-1000 mg dose level). Induction of δ-aminolevulenic acid synthetase (800 mg dose level).	Poland 1971

Administered by gavage or capsule.

TABLE 4--DICHLOROBENZENES (cont.)
SUBCHRONIC INHALATION TOXICITY STUDIES

Compound	Species	Dose and Duration	Effect	Ref.
1,4-Dichlorobenzene	Rabbits	4.6-4.8 mg/l; 62 exposures (8 hr. each) over 83 days	Tremors, weakness, death, lateral nystagmus and transitory edema of cornea and optic nerve head.	Pike 1944
1,4-Dichlorobenzene	Rats, Rabbits, Guinea Pigs	100 mg/l for 20-30 min/day for up to 34 days	Extensive renal damage, swollen and degenerated uriniferous tubule epithelium, hyperemic and pyknotic glomeruli, tremors, twitches, "mark time" reflex, loss of righting reflex, nystagmus.	Zupko and Edwards 1949
1,4-Dichlorobenzene	Guinea Pigs	4.8 mg/l, 8 hrs./day, 23 exposures	Liver degeneration and necrosis, CNS effects, 2/16 died.	Hollingsworth 1956
1,4-Dichlorobenzene	Rats	4.8 mg/l, 8 hrs./day, 69 exposures	Liver degeneration and necrosis, renal tubule epithelium swelling, CNS effects, 4/34 died.	Hollingsworth 1956
1,4-Dichlorobenzene	Rabbits	4.8 mg/l, 8 hrs./day, 62 exposures	Liver degeneration and necrosis, CNS effects, 4/16 died.	Hollingsworth 1956
1,4-Dichlorobenzene	Rabbits, Guinea Pigs	(Undetermined) up to 1 month	Death, liver cell vacuolation and necrosis.	Berliner 1939

TABLE 4--DICHLOROBENZENES (cont.)
SUBCHRONIC AND CHRONIC DIETARY TOXICITY STUDIES

Compound	Species	Dose and Duration	Effect	Ref.
1,2-Dichlorobenzene	Rats	455 mg/kg/day for 15 days	Loss of weight, increased liver and urinary coproporphyrin, urinary porphobilinogen, fatty and necrotic liver damage.	Rimington, Ziegler 1963
1,2-Dichlorobenzene	Rats	81, 244 or 471 mg/kg/day for 28 days	Pale kidneys, mottled livers (highest dose).	Monsanto 1965a
1,4-Dichlorobenzene	Rats	70, 241 or 482 mg/kg/day for 28 days	Pale kidneys, mottled livers (all dose levels).	Monsanto 1965b
1,4-Dichlorobenzene	Rats	770 mg/kg/day for 5 days	Loss of weight, increased liver and urinary uroporphyrin, urinary coproporphyrin, porphobilinogen, delta-aminolevulinic acid; non-necrotic liver cell degeneration.	Rimington, Ziegler 1963

SUBCHRONIC PARENTERAL TOXICITY STUDIES

Compound	Species	Dose and Duration	Effect	Ref.
1,4-Dichlorobenzene	Guinea Pigs	125 and 250 mg/day for 10 days (intramuscular)	Loss of weight, liver steatosis, reduced hepatic and muscular glycogen.	Frada, Cali 1958
1,4-Dichlorobenzene	Guinea Pigs	125 mg/day for 20 days	Reduced activity of the prothrombin complex and thrombokinase, attributed to liver damage.	Salamone, Coppola 1960
1,4-Dichlorobenzene	Guinea Pigs	125 mg/day for 20 days	Decreased acid and alkaline phosphatase in liver, kidney and brain, increased liver and muscle fat, reduced liver and muscle glycogen.	Cali 1960

TABLE 4--DICHLOROBENZENES (cont.)
SUBCHRONIC PARENTERAL TOXICITY STUDIES

Compound	Species	Dose and Duration	Effect	Ref.
1,4-Dichlorobenzene	Guinea Pigs	125 mg/day i.m. for 21 days	Increased reaction and clot-formation times.	Coppola 1963
1,4-Dichlorobenzene	Guinea Pigs	125 mg/day i.m. for 20 days	Weight loss, increased blood serum transaminase.	Totaro 1964

Table 5--TRICHLOROBENZENES, TETRACHLOROBENZENES, AND PENTACHLOROBENZENE SUBCHRONIC INHALATION TOXICITY STUDIES

Compound	Species	Dose and Duration	Effect	Ref.
1,2,4-Trichlorobenzene	Rats	25.3, 49.2 and 92.8 ppm for 26 weeks	Microscopic changes in parenchymal cells of liver and kidney.	Coate et al 1977

SUBCHRONIC AND CHRONIC DIETARY TOXICITY STUDIES

Compound	Species	Dose and Duration	Effect	Ref.
1,2,3-Trichlorobenzene	Rats	785 mg/kg/day for 7 days	Loss of weight, increased urinary coproporphyrin, porphobilinogen, non-necrotic liver cell degeneration.	Rimington, Ziegler 1963
1,2,4-Trichlorobenzene	Rats	730 mg/kg/day for 15 days	Loss of weight, increased urinary coproporphyrin, uroporphyrin, porphobilinogen, delta-aminolevulinic acid; fatty and necrotic liver damage.	Rimington, Ziegler 1963
1,2,3,4-Tetrachlorobenzene	Rats	660 mg/kg/day for 10 days	Loss of weight, increased liver and urinary coproporphyrin, uroporphyrin, urinary porphobilinogen, delta-aminolevulinic acid; non-necrotic liver cell degeneration.	Rimington, Ziegler 1963
1,2,4,5-Tetrachlorobenzene	Rats	905 mg/kg/day for 5 days	No effect on urinary porphyrin excretion; non-necrotic liver cell degeneration.	Rimington, Ziegler 1963
1,2,4,5-Tetrachlorobenzene	Dogs	5 mg/kg/day for 2 years	Elevated SAP and total bilirubin.	Braun et al 1978
Pentachlorobenzene	Rats	32, 62, 134 mg/kg/day	No clinical effects. Liver and hepatocellular enlargement; increased kidney weight. Suckling pups developed tremors and died.	Linder et al 1978

Table 5--TRICHLOROBENZENES, TETRACHLOROBENZENES, AND PENTACHLOROBENZENE (cont.)
ORAL* SUBCHRONIC TOXICITY STUDIES

Compound	Species	Dose and Duration	Effect	Ref.
1,2,4,5-Tetrachlorobenzene	Rats	75 mg/kg, 2 months	Decreased liver function, erythremia.	Fomenko 1965
1,2,4,5-Tetrachlorobenzene	Rabbits	0.005 or 0.05 mg/kg/day for 6-8 months	Increased retention of galactose in the blood, glycogen formation disorder. Hemoglobin increase and reticulocytosis (0.05 mg dose level).	Fomenko 1965
1,2,4,5-Tetrachlorobenzene	Rats	0.005 or 0.05 mg/kg/day for 6-8 months	Slower performance of conditioned response.	Fomenko 1965

*Administered by gavage or capsule

c. Other Indicators of Systemic Toxicity

(1) Metabolism

The metabolic pathways of benzene, monochlorobenzene, dichlorobenzenes, trichlorobenzenes, tetrachlorobenzenes, pentachlorobenzene, and hexachlorobenzene are treated sequentially in the following text and in Figures 1-7. These metabolic reactions represent classic conversions of chlorinated aromatic compounds in the liver.

(a) Benzene

Benzene is absorbed readily from the gastrointestinal tract. Rusch et al. (1977) concluded from the available experimental findings that the metabolism and elimination of benzene in humans and in all animal species studied follow very similar pathways (Figure 1). About 40 percent is eliminated unchanged in the expired air. The remainder is oxidized to benzene oxide by the aryl hydrocarbon hydroxylase (AHH) enzyme system. The benzene oxide then breaks down via one of three pathways:

(i) It can rearrange spontaneously to yield phenol by the "NIH shift". The majority of the phenol formed subsequently conjugates with glucuronic and sulfuric acids. However, some of the phenol undergoes further oxidation to hydroquinone.

(ii) Benzene oxide can be acted upon by epoxide hydrase to give benzene glycol, which then yields predominantly catechol via enzymatic dehydrogenation, and some trans-trans-muconic acid following subsequent oxidation. The majority of the catechol is conjugated and eliminated with a small amount being oxidized to hydroxyhydroquinone.

(iii) The benzene oxide can react with glutathione in the presence of an epoxidetransferase, followed by dehydration to produce phenylmercapturic acid.

(b) Monochlorobenzene

Monochlorobenzene resembles benzene with regard to absorption, excretion and metabolism. It is absorbed easily from the gastrointestinal tract and excreted in the urine (Smith et al. 1950, Spencer and Williams 1950, Azouz et al. 1953). Unchanged monochlorobenzene is eliminated with expired air (25-30 percent), though not as extensively as benzene (40 percent). As illustrated in Figure 2, monochlorobenzene undergoes three primary enzymic attacks in the production of the major urinary metabolites, 4-chlorocatechol and 4-chlorophenylmercapturic acid. 3-Chlorocatechol and the three isomeric chlorophenols are produced as minor metabolites.

(i) Formation of 3-chlorobenzene oxide which either isomerizes to 2-chlorophenol or goes to 2,3-dihydro-2,3-dihydroxychlorobenzene via epoxide hydrase and finally forms 3-chlorocatechol (Spencer and Williams 1950, Selander et al. 1975 a,b).

(ii) Formation of 3-chlorophenol via a direct oxidative pathway (Selander et al. 1975a).

(iii) Formation of 4-chlorobenzene oxide which either conjugates with glutathione to produce 4-chlorophenylmercapturic acid (Smith et al. 1950, Azouz et al. 1953, Parke and Williams 1955, Williams 1959, 1975), rearranges to 4-chlorophenol (Spencer and Williams 1950, Smith et al. 1950, Selander 1975a), or is converted to 3,4-dihydro-3,4-dihydroxychlorobenzene by the action of epoxide hydrase and finally to 4-chlorocatechol via enzymic dehydrogenation (Azouz et al. 1953, Smith et al. 1950, Williams 1959, Williams et al. 1975).

(c) Dichlorobenzene

Dichlorobenzenes are absorbed less readily than are benzene and monochlorobenzene. Metabolic attack upon at least the 1,2- and 1,3-isomers of this compound appears to involve the formation of arene oxides by epoxidase (Figure 3). Unlike monochlorobenzene, however, mercapturic acids and catechols are not major metabolites of dichlorobenzenes. They are formed merely as minor metabolites of 1,2- and 1,3-dichlorobenzenes and are not formed at all by the 1,4-isomer (Azouz et al. 1953, 1954, Parke and Williams 1955). The major metabolites of 1,2- and 1,3-dichlorobenzene are 3,4- and 2,4-dichlorophenol respectively, which are excreted as conjugates of glucuronic and sulfuric acids. 1,4-Dichlorobenzene is mainly oxidized to 2,5-dichlorophenol and 2,5-dichloroquinol (Azouz et al. 1954, 1955).

(d) Trichlorobenzenes

Trichlorobenzenes are absorbed and excreted slowly. As indicated in Figure 4, these compounds are metabolized initially to arene oxides and are excreted in urine mainly as trichlorophenols. The major metabolite of the 1,2,3-isomer is 2,3,4-trichlorophenol, but small amounts of 3,4,5-trichlorocatechol and 3,4,5- and 2,3,6-trichlorophenols are also formed (Jondorf et al. 1954, 1955b, Kohli et al. 1976).

1,2,4-Trichlorobenzene is mainly metabolized to 2,4,5- and 2,3,5-trichlorophenols. They are excreted together with small amounts of 3,4,6-trichlorocatechol, and 2,3,5- and 2,4,5-trichlorophenylmercapturic acids.

2,3,5-Trichlorophenol and 2,4,6-trichlorophenol were identified as the major metabolites of 1,3,5-trichlorobenzene, which was also shown to dechlorinate and produce 4-chlorophenol and 4-chlorocatechol as minor urinary metabolites (Kohli et al. 1976, Parke and Williams 1960, Jondorf et al. 1954, 1955a).

(e) Tetrachlorobenzenes

Tetrachlorobenzenes are absorbed with still greater difficulty. They are excreted slowly and yield no mercapturic acids or catechols. 1,2,3,4-Tetrachlorobenzene gives two phenolic metabolites, 2,3,4,6- and 2,3,4,5-tetrachlorophenol which can be generated from 2,3,4,5-tetrachlorobenzene, oxide (Jondorf et al. 1958, Kohli et al. 1976).

1,3,4,5- and 2,3,4,6-Tetrachlorobenzene oxides are the proposed intermediates in the conversion of 1,2,3,5-tetrachlorobenzene into 2,3,4,5-, 2,3,4,6-, and 2,3,5,6-tetrachlorophenols (Jondorf et al 1958, Kohli et al. 1976). All phenols are derived via the "NIH shift".

1,2,4,5-Tetrachlorobenzene gives only one metabolite, 2,3,5,6-tetrachlorophenol. There is evidence that this isomer is also partly dechlorinated in the gut to less chlorinated benzenes (Jondorf et al. 1958, Kohli et al. 1976).

(f) Pentachlorobenzenes

Pentachlorobenzene is absorbed poorly from the gastrointestinal tract and is removed slowly from the tissues. Its major metabolites are pentachlorophenol and 2,3,4,5-tetrachlorophenol (Kohli et al. 1976, Koss and Koransky 1978). As indicated in Figure 6, pentachlorophenol can be formed by direct oxidation, but formation of 2,3,4,5-tetrachlorophenol may involve reductive dechlorination and the production of an arene oxide intermediate. Other metabolites identified include tetrachloroquinone, 2,3,5,6-tetrachlorophenol and a hydroxylated chlorothiocompound (Koss and Koransky 1978). The mechanism of formation of these metabolites, however, has not been established.

(g) Hexachlorobenzene

Hexachlorobenzene is very poorly absorbed from the gastrointestinal tract, is excreted predominantly in feces, and is removed very slowly from tissues. The mechanism of formation of the known metabolites of hexachlorobenzene is unclear. Like pentachlorobenzene, it undergoes reductive dechlorination since metabolites containing only three, four, or five chlorine atoms have been identified (Figure 7). The urinary metabolites present at the highest levels were identified as pentachlorophenol, tetrachlorohydroquinone, and pentachlorothiophenol (Cooper 1978, Koss et al. 1976, Rozman et al. 1977, Yang et al. 1975, Lui and Sweeney 1975). Small amounts of 2,3,4,6- and 2,3,5,6-tetrachlorophenols and traces of 2,3,4,-, 2,4,5- and 2,4,6-trichlorophenols are also present (Mehendale et al. 1975, Engst et al. 1976, Renner and Schuster 1977, Cooper 1978).

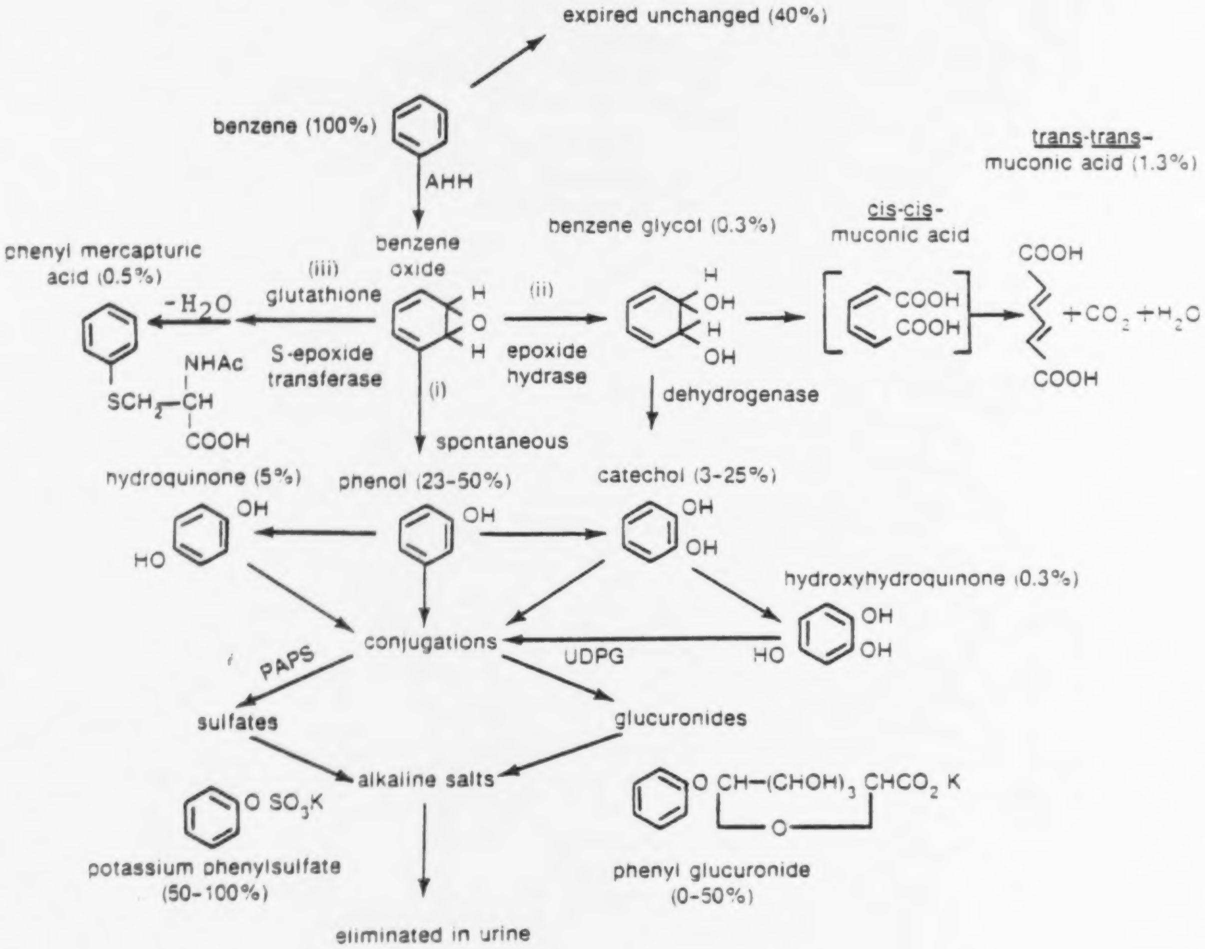
Discussion of Metabolism Data. Several points should be kept in mind in evaluating metabolism data on aromatic compounds. The formation of phenolic metabolites can occur by more than one mechanism and, therefore, does not by itself show that arene oxide intermediates are involved (Jerina and Daly 1976). Such intermediates can be suspected if the product phenol appears to be the result of a ring substituent shift to an adjacent position (the "NIH shift") or if certain other metabolites--1,2-dihydrodiols, catechols, or arylmercapturic acids--are detected. In some cases, the intermediacy of an arene oxide can be demonstrated only by more sophisticated experiments involving isotopically-labeled starting material or chemically synthesized arene oxides, and such experiments may be crucial in determining how much of a given phenol is formed by arene oxide and how much by nonarene oxide pathways (Jerina and Daly 1976). Authors of metabolism papers sometimes suggest uncritically an arene oxide pathway where the evidence for this is ambiguous or nonexistent. In other cases the intermediacy of arene oxides is a possibility but not demonstrable from the available data; that is, the evidence is neither for nor against this pathway for the compound in question. In Figures 1-7 arene oxide intermediates proposed in the literature without sufficient evidence are indicated by a question mark below the structure.

Another problem encountered with much published metabolism data is the failure to account for all of the administered compound. Among published reports on chlorinated benzenes, the quantity accounted for ranges from 85% (Parke and Williams 1960) to as little as 2% (e.g., Kohli et al. 1976). For the higher chlorinated benzenes in particular, much of the missing chemical may have been simply excreted unchanged, but the lack of a materials balance leaves open the question of whether a significant metabolic pathway has escaped detection. These shortcomings merely reflect the incompleteness of the available data and do not preclude some generalization on the basis of the data in hand.

Thus, it is clear from the metabolism data and from Figures 1-7 that the patterns of absorption, excretion, and metabolism of the chlorinated benzenes shift with the degree of chlorination, with monochlorobenzene more closely resembling benzene and pentachlorobenzene more closely resembling hexachlorobenzene. It is noteworthy that in general mono-, di-, tri-, and tetrachlorobenzenes can be metabolized initially to arene oxides, compounds that can bind covalently to vital cellular macromolecules, which may initiate oncogenesis, teratogenesis, or mutagenesis. Another potentially toxic effect of the metabolism of chlorinated benzenes is the formation of tumor promoters. 2-Chlorophenol and 3-chlorophenol, metabolites of monochlorobenzene, and 2,4,5-trichlorophenol, a metabolite of 1,2,4-trichlorobenzene, are known to promote oncogenesis [see Section III.G.1.c.(4) for more discussion].

Although there appears to be only a single study of the metabolism of a chlorinated benzene in humans, the data are valuable because other mammalian species were investigated for comparison. Monochlorobenzene was metabolized to the same major metabolites (i.e., 4-chlorophenol, 4-chlorocatechol, and 4-chlorophenylmercapturic acid) in man and in 12 other mammalian species (Williams et al. 1975). This suggests that, at least for this chlorinated benzene, metabolism studies performed in lower species are relevant to man.

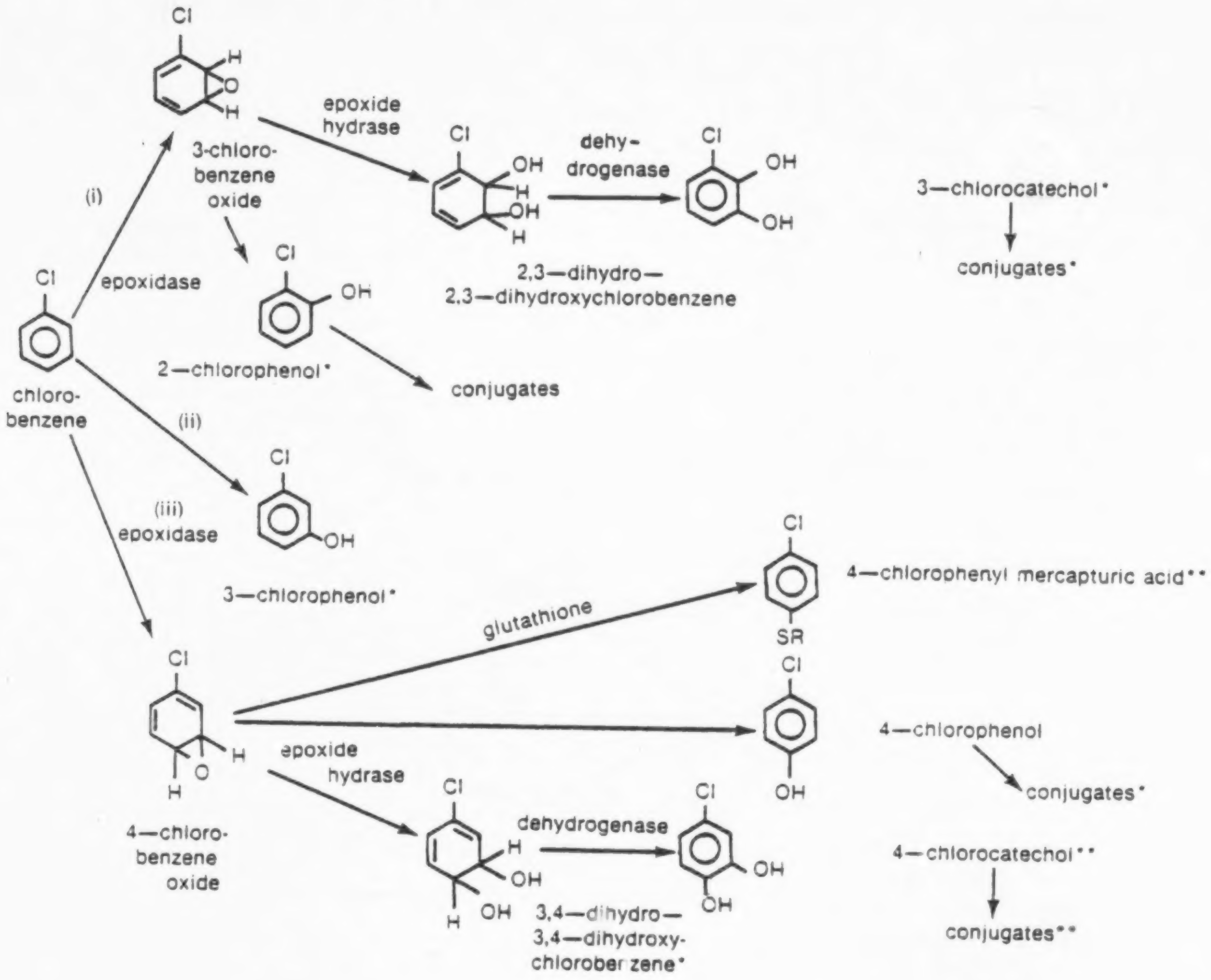
FIGURE 1
PATHWAYS OF BENZENE METABOLISM AND ELIMINATION

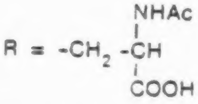


Reference: Rusch *et al.* (1977)

AHH = aryl hydrocarbon hydroxylase
UDPG = uridine diphosphate glucuronyl transferase
PAPS = 3′-phospho-adenosin-5′-phosphosulfate

FIGURE 2
METABOLISM OF MONOCHLOROBENZENE





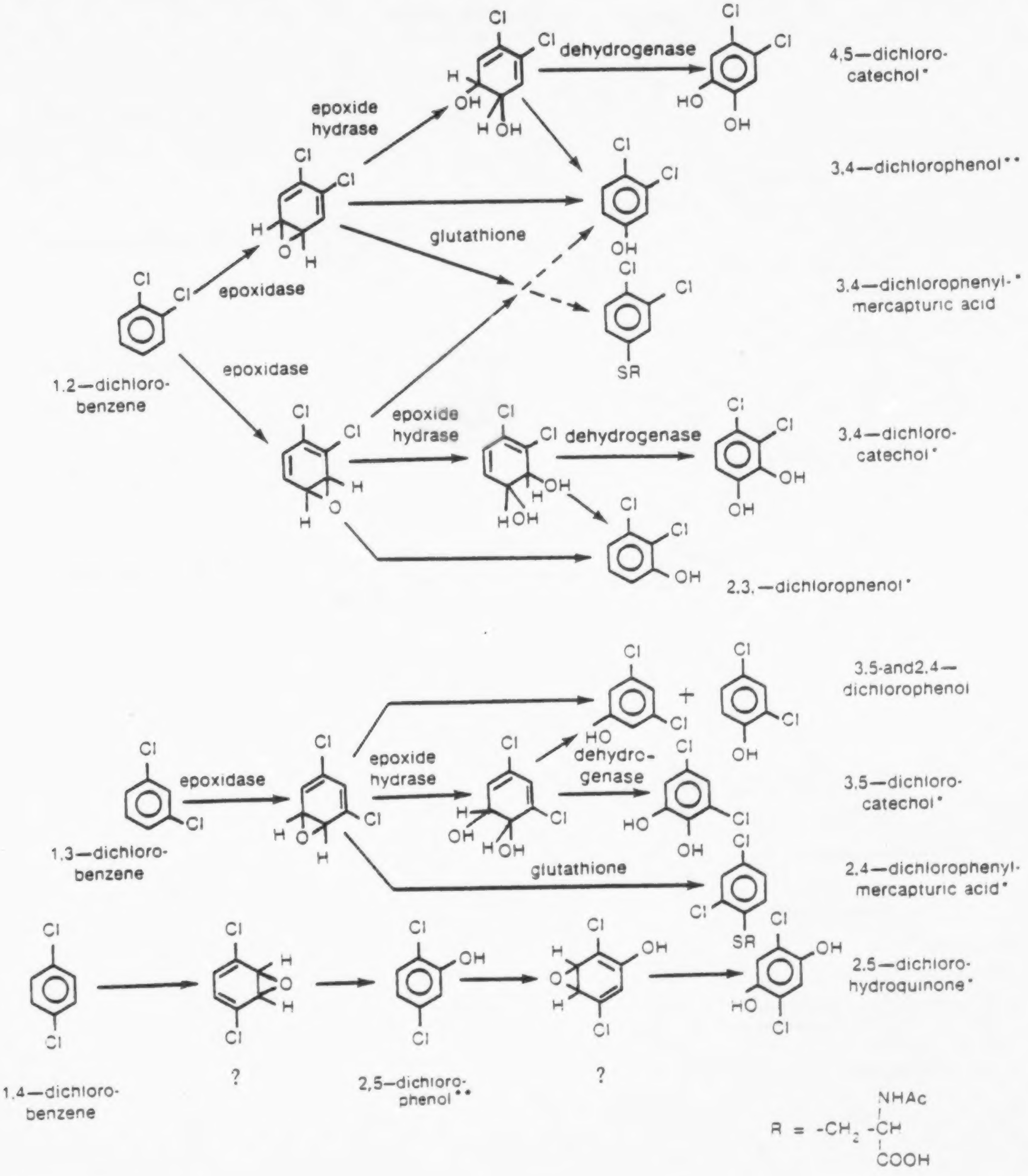
$$R = -CH_2-\overset{\displaystyle NHAc}{\underset{\displaystyle COOH}{CH}}$$

* Urinary metabolite

**Major urinary metabolite

References: Spencer and Williams (1950); Smith et al. (1950); Azouz et al. (1953); Parke and Williams (1955); Williams (1959); Lindsay-Smith et al. (1972); Selander et al. (1975a, 1975b); Williams et al. (1975).

FIGURE 3
METABOLISM OF DICHLOROBENZENES



* Urinary metabolite

**Major urinary metabolite

References: Azouz et al. (1953, 1954, 1955); Parke and Williams (1955).

FIGURE 4
METABOLISM OF TRICHLOROBENZENES

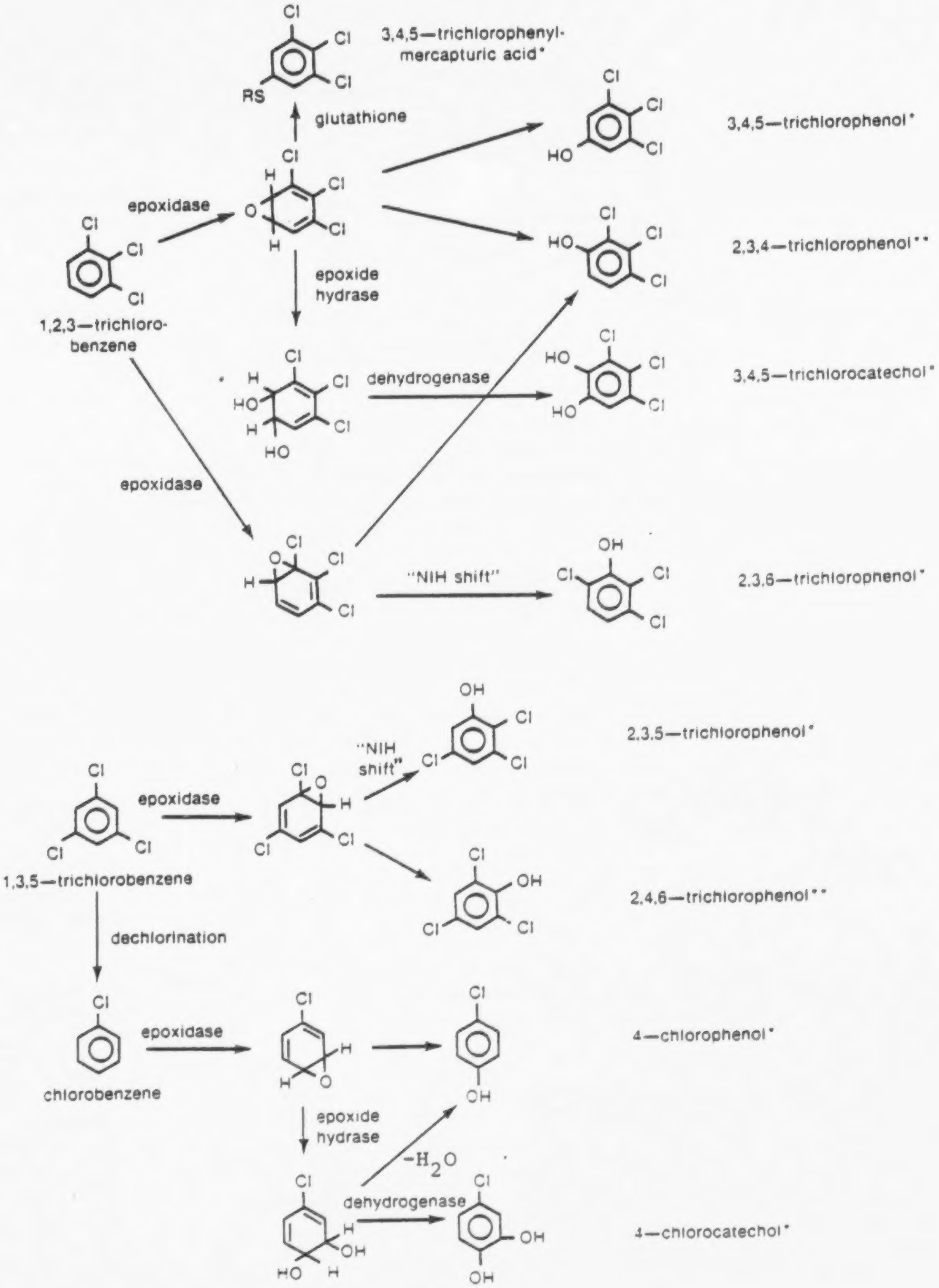
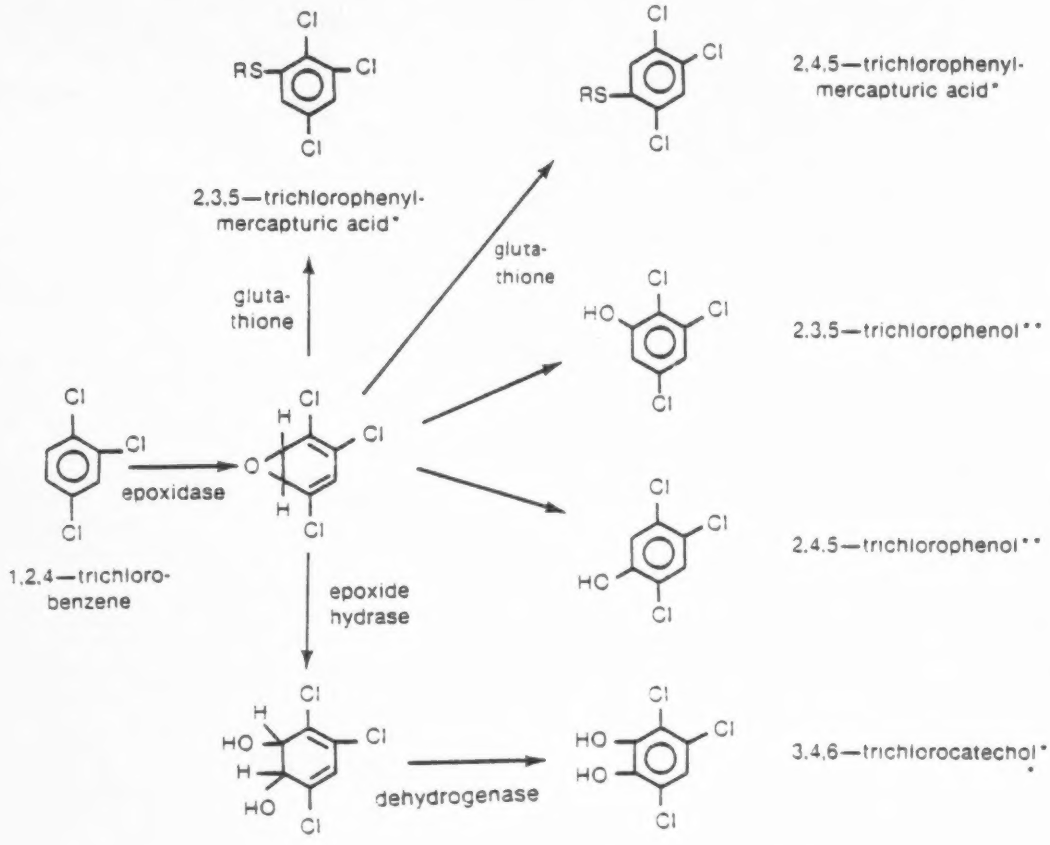


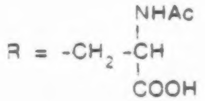
FIGURE 4
METABOLISM OF TRICHLOROBENZENES (CONTINUED)



*Urinary metabolite

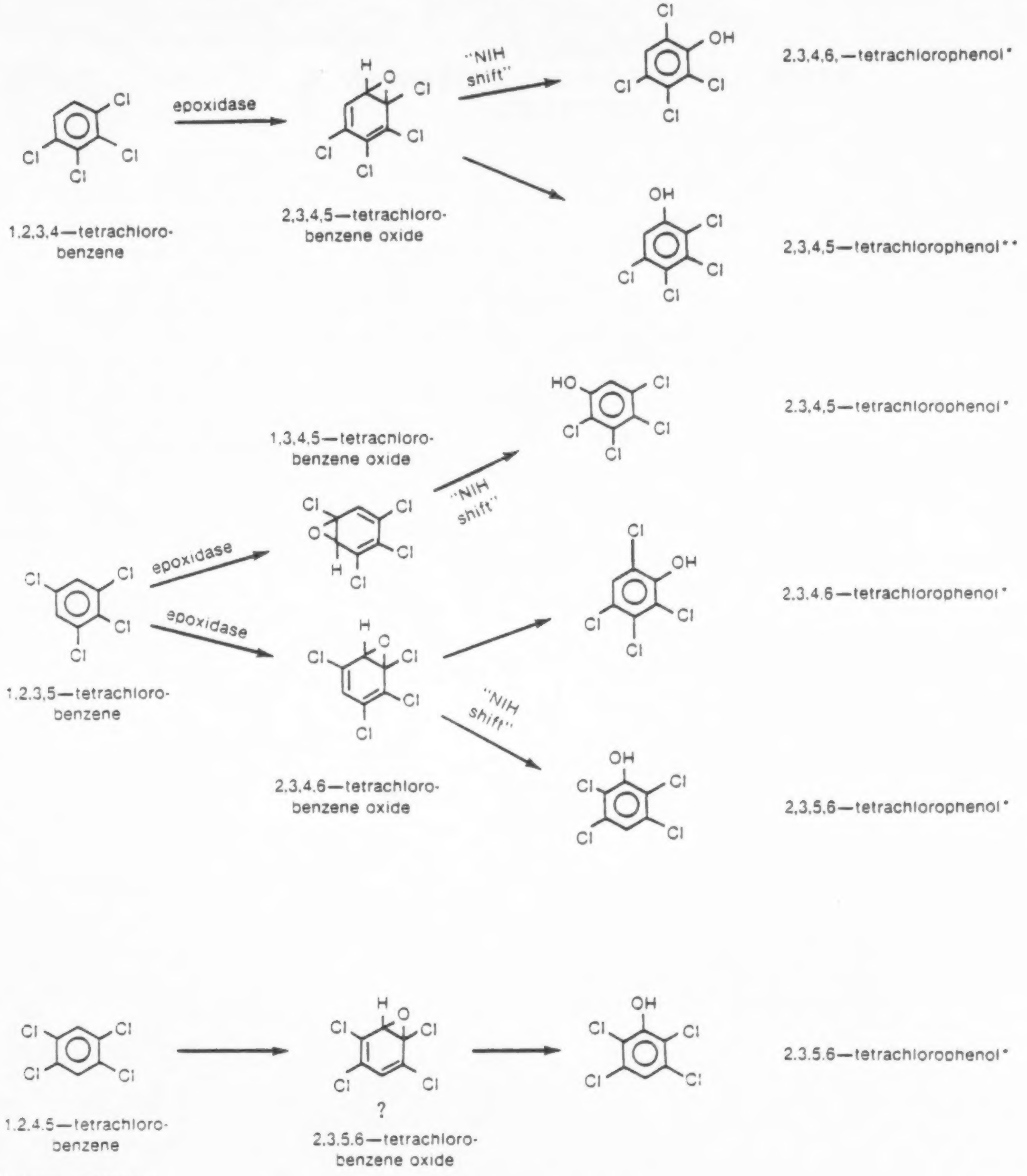
**Major urinary metabolite

References: Jondorf et al. (1954, 1955a, 1955b); Parke and Williams (1960); Kohli et al. (1976).



$R = -CH_2-\underset{COOH}{\overset{NHAc}{CH}}$

FIGURE 5
METABOLISM OF TETRACHLOROBENZENES

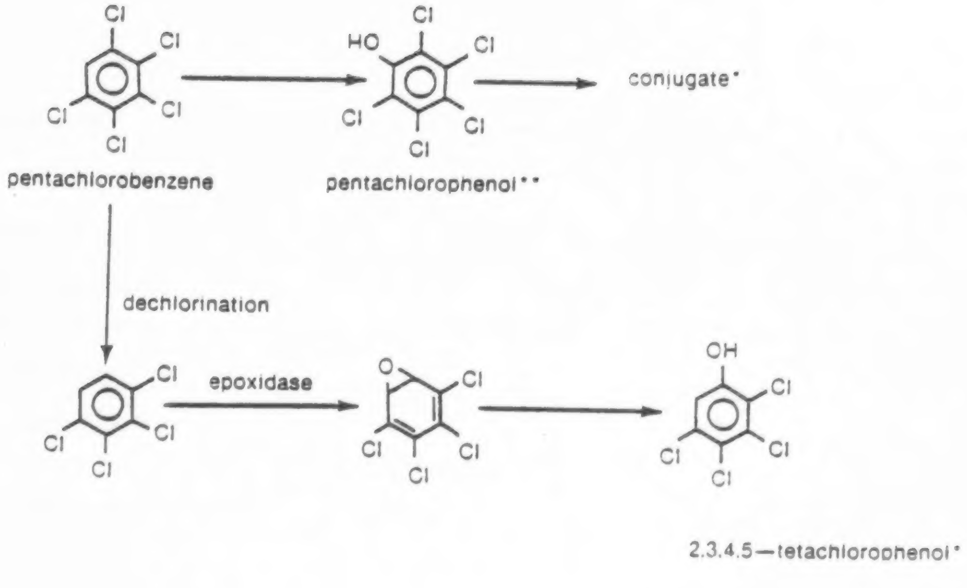

epoxidase
"NIH shift"
1,2,3,4—tetrachloro-benzene
2,3,4,5—tetrachloro-benzene oxide
2,3,4,6,—tetrachlorophenol*
2,3,4,5—tetrachlorophenol**
1,3,4,5—tetrachloro-benzene oxide
epoxidase
epoxidase
"NIH shift"
"NIH shift"
1,2,3,5—tetrachloro-benzene
2,3,4,6—tetrachloro-benzene oxide
2,3,4,5—tetrachlorophenol*
2,3,4,6—tetrachlorophenol*
2,3,5,6—tetrachlorophenol*
1,2,4,5—tetrachloro-benzene
?
2,3,5,6—tetrachloro-benzene oxide
2,3,5,6—tetrachlorophenol*
Cl
OH
HO
H
O

*Urinary metabolite

**Major urinary metabolite

References: Jondorf et al. (1958); Kohli et al. (1976).

FIGURE 6
METABOLISM OF PENTACHLOROBENZENE

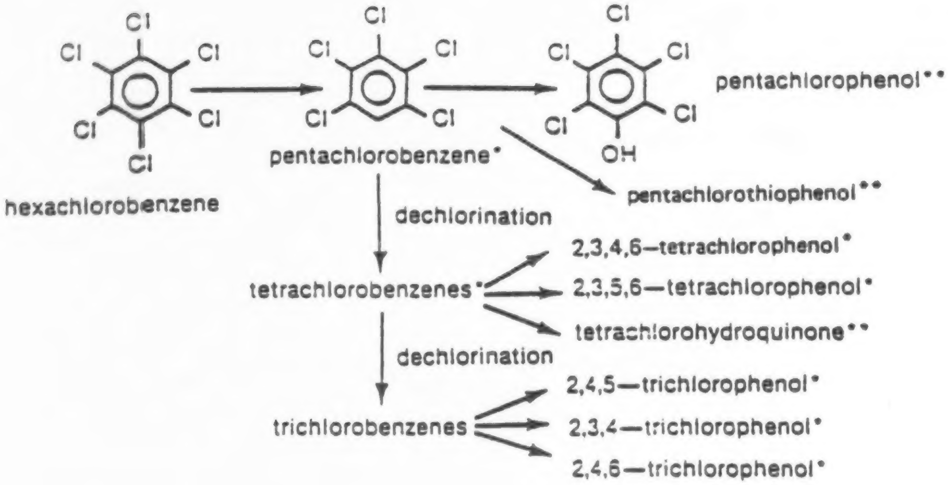


*Urinary metabolite

**Major urinary metabolite.

Reference: Kohli *et al.* (1976); Leber *et al.* (1977); Koss and Koransky (1978).

FIGURE 7
METABOLISM OF HEXACHLOROBENZENE



*Urinary metabolite

**Major urinary metabolite

References: Cooper (1978); Engst *et al.* (1976); Kohli *et al.* (1976); Koss and Koransky (1978); Koss *et al.* (1976, 1978a, 1978b); Menendale et al. (1975); Rozman *et al.* (1977, 1978; Renner and Schuster (1977); Bedford (1979); Yang *et al.* (1975); Lui and Sweeney (1975).

(2) Porphyria

Porphyria is any one of a group of diseases that result from a disturbance of porphyrin metabolism, usually in the liver, which leads to an increase in the tissue and circulating blood levels of porphyrins and related compounds.

Hexachlorobenzene consumption produces hepatic porphyria in humans, with cutanea tarda lesions and porphyrinuria (Schmid 1960). This effect can also be produced in rats, rabbits, guinea pigs and mice (Ockner and Schmid 1961, DeMatteis et al. 1961, San Martin de Viale et al. 1970, Taljaard et al. 1972, Rajamanickam et al. 1972, Stonard 1974). Some of the lower chlorinated benzenes will also affect porphyrin metabolism, but not to the same extent. Rimington and Ziegler (1963) found that high doses (455-1140 mg/kg) of monochloro-, ortho- and para-dichloro-, 1,2,3- and 1,2,4-trichloro-, and 1,2,3,4-tetrachlorobenzene given to rats for brief periods resulted in porphyria which varied in intensity and expression from compound to compound. 1,2,4,5-Tetrachlorobenzene (905 mg/kg) did not affect liver or urinary porphyrins. The increases in urinary porphyrin excretion, caused by other chlorinated benzenes, were fairly small considering the large doses administered. Even the substantial increase in uroporphyrin excretion seen after para-dichlorobenzene administration is modest (10 ug/day) when compared with the huge increases following hexachlorobenzene poisoning (100-400 ug/day) (Ockner and Schmid 1961).

Carlson (1977) compared para-dichlorobenzene, 1,2,4- trichlorobenzene and hexachlorobenzene in their ability to induce porphyria in rats. Doses of 50 mg/kg/day of hexachlorobenzene (p.o.) for 30 days in female rats (chosen because of their greater susceptibility to the porphyrinogenic effects of hexachlorobenzene) caused a statistically significant increase in both liver and urine porphyrins. However, doses of up to 200 mg/kg/day for 120 days of para-dichlorobenzene and 1,2,4-trichlorobenzene caused no porphyrinuria and only minor changes in the porphyrin content of the liver. It is possible that the porphyrinuria seen acutely with large doses (Rimington and

Ziegler 1963) represents massive liver damage, rather than porphyria. In addition, although Rimington and Ziegler noted skin lesions with 1,2,3,4-tetrachlorobenzene, they were not the cutanea tarda lesions seen in cases of hexachlorobenzene-induced porphyria, as they were not a photosensitive response, but were apparently similar to chloracne. The large doses used by Rimington and Ziegler, in some cases, approach the LD_{50} for the compounds, while very small amounts of hexachlorobenzene, which has a high LD_{50}, suffice to initiate porphyria, a further indication that liver damage, rather than a specific effect on porphyrin metabolism, may be the cause of the effects seen by Rimington and Ziegler (1963). The spectrum of porphyrins excreted (coproporphyrin and porphobilinogen) is also different from that seen in hexachlorobenzene poisoning (7- and 8-carboxyporphyrin). While it is possible that the porphyrias induced by hexachlorobenzene and the lower chlorinated benzenes are similar and have a common mechanism, it seems more likely that they result from different causes.

(3) Structural Relationships

The chlorinated benzenes are structurally related to benzene and hexachlorobenzene, both of which cause chronic effects (Schmid 1960, Aksoy et al. 1971, 1972, 1976, Tareeff et al. 1963), as discussed below. The oncogenicity of benzene and hexachlorobenzene are discussed in Section III.G.1.c.(2).

(a) Benzene

Benzene has been shown to produce damage to the hematopoietic system (Aksoy et al. 1971). Leukopenia, thrombocytopenia, and pancytopenia are among the health effects reported among workers who were chronically exposed to the solvent. Similar effects have been reported in dogs (Svirbely et al. 1944, Hough et al. 1944), rats, rabbits, and guinea pigs (Wolf et al. 1956, Deichmann et al. 1963). These effects appear similar to those such as thrombocytopenia, leukopenia, and aplastic anemia among other blood effects attributed to chlorinated benzenes and discussed earlier in this subchronic effects section.

Since it is unknown what aspect of benzene metabolism is responsible for the observed effects, or even whether there is a single cause of all of benzene's effects, the significance of the structural similarity with chlorinated benzenes is uncertain. Until more information is available, this structural relationship remains only suggestive of the potential of chlorinated benzenes to cause similar toxicity.

(b) Hexachlorobenzene

Hexachlorobenzene has been shown to produce histopathological changes in the liver and spleen in rats (Kuiper-Goodman 1977). Neutrophilia and some hepatotoxicity were found in beagle dogs after exposure to hexachlorobenzene (Gralla and Fleischman 1976). All of the chlorinated benzenes tested induced similar liver damage.

2. Decision

An assessment of the literature to date reveals that most of the chlorinated benzenes that have been tested thus far produce similar health effects. Mono- through tetrachlorobenzenes have been noted for their effects on the liver and the hematopoietic system in humans and animals (Varshavskaya 1967, Monsanto 1967a, Hollingsworth et al. 1956, 1958, Coate et al. 1977, Fomenko 1965, Cotter 1953, Campbell and Davidson 1970). Mono-, di- and trichlorobenzenes have been shown to produce variable changes in the kidney (Khanin 1977, Monsanto 1978g, Hollingsworth et al. 1956, 1958, Coate et al. 1977). The results of an unpublished subchronic study indicate that pentachlorobenzene also induces effects in the liver and kidney (Linder et al. 1980). Although the experimental designs of some of these tests have weaknesses, the test results lead to the common conclusion that the chlorinated benzenes produce similar chronic health effects and may present an unreasonable risk to human health.

Determination of the most sensitive species, route of administration, and health effect cannot be done because of conflicting results from the studies reported in the literature. For example, for monochlorobenzene the Monsanto results (1978g) indicate that the dog is more sensitive via the inhalation route than the rat, but Khanin (1977) observed effects in rats at doses considerably lower than those used in the Monsanto study. Another Monsanto study (1967a and 1967b) indicates that the dog is more sensitive via the oral route than the rat, but Varshavskaya (1967) observed effects at doses considerably lower than those used in this Monsanto study as well. A similar discrepancy occurs with _o_-dichlorobenzene (Varshavskaya 1967, Hollingsworth et al. 1958).

The chlorinated benzenes are structurally related to two compounds with recognized chronic effects: benzene and hexachlorobenzene. Benzene has been noted for its effects on the hematopoietic system in both humans and animals (Aksoy et al. 1971, Svirbely et al. 1944, Hough et al. 1944, Wolf et al. 1956). Chlorinated benzenes may produce similar effects (Perrin 1941, Petit and Champeix 1948, Wallgren 1953, Monsanto 1978g). Hexachlorobenzene has been shown to produce changes in the liver and spleen (Schmid 1960, Kuiper-Goodman 1977). Chlorinated benzenes also damage these organs (Monsanto 1967a, Hollingsworth et al. 1956, 1958, Coate et al. 1977, Fomenko 1965).

Both monochlorobenzene and its analog monobromobenzene are metabolized to arene oxides. Bromobenzene has been found to bind covalently to liver proteins and to produce liver necrosis, particularly after depleting 90 percent of hepatic glutathione via formation of mercapturic acid metabolites (Reid and Krishna 1973, Jollow et al. 1974). Mono-, di- and trichlorobenzenes (except _p_-dichlorobenzene, for which the evidence is ambiguous) are metabolized to arene oxides and excreted partly as mercapturic acids (see Figures 2, 3, and 4, Metabolism Section), indicating their potential to react with proteins and possibly cause necrosis. This may explain the liver damage associated with all of the chlorinated benzenes tested and the fact that monochlorobenzene has been reported to cause renal necrosis (Reid 1973).

In summary, the subchronic studies reported in the literature show that the chlorinated benzenes produce similar chronic health effects when given by several routes of administration. These compounds are structurally related to two compounds of known chronic toxicity: benzene and hexachlorobenzene. They are metabolized to reactive compounds that may bind to macromolecules in the cell and thus produce a variety of toxic effects. Available information does not permit the Agency to complete an assessment of the nature and extent of chronic toxicity risks associated with exposure to the chlorinated benzenes because the reports in the literature give insufficient detail or apparently inconsistent results.

3. Proposed Testing: Subchronic and Chronic Effects Testing

EPA proposes that the chlorinated benzenes, except pentachlorobenzene, be tested for chronic health effects. Pentachlorobenzene has already undergone adequate subchronic testing.

A number of reviewers have suggested that short-term (90 day) animal studies can in some cases gives reasonable indications of the long-term (lifetime) effects of chemicals and that long-term studies therefore need not always be performed (Weil and McCollister 1963, Peck 1968, McNamara 1976). On the basis of the test data available for chlorinated benzenes, EPA believes that the nature and degree of chronic effects induced by chlorinated benzenes can be determined in 90-day subchronic toxicity studies following the test standards proposed in the Federal Register on July 26, 1979 (USEPA 1979c).

EPA has proposed standards recommending the use of a rodent and a nonrodent species, with the dog as the preferred nonrodent. Because the studies by Khanin (1977) and Varshavskaya (1967) indicate that the rat may be the more sensitive species (see above under "Decision"). EPA is proposing that the 90-day subchronic testing be done only in rats. The results of this study will assist the Agency in determining whether a safe level of human exposure can be established for these effects.

The Agency is aware that a 90-day inhalation study, of which only an abstract has been published (Watanabe et al. 1978), has been performed on 1,2,4-trichlorobenzene in rats that may suffice to characterize the subchronic toxicity of the compound. EPA will try to obtain more details of this study.

4. Testing Under Consideration: Metabolism Studies

EPA believes that valuable information would be obtained from studies designed (a) to determine the distribution of chlorinated benzenes to tissues and organs of one species, (b) to learn the rates of their clearance from these tissues, and (c) to ascertain whether or not chlorinated benzenes or their metabolites form covalent compounds with macromolecules, particularly in the brain and gonads and in organs from which excretion is slow. If any of these compounds do form covalent compounds with macromolecules, experiments should determine further whether binding is to DNA, protein, or both. EPA believes that this information could strengthen the basis for using the results from tested chlorobenzenes to characterize the category (see Section IV of this document). EPA is not proposing specific metabolism studies at this time since the Agency now has standards only for studies on absorption and excretion. EPA is interested in comments from all sectors on what other kinds of metabolism studies are needed.

C. Neurotoxic Effects

1. Evaluation of Pertinent Studies

a. Human Case Reports

Reich (1934) reported an instance of a two-year-old male who swallowed an estimated 5 to 10 ml of monochlorobenzene. Within two hours, the child was pale, his lips cyanotic, and he had no detectable reflexes to strong stimuli. When hospitalized, he was unconscious and cyanotic, with twitching in the head and neck regions. The child began to regain consciousness after three hours, and all signs were normal within eight hours. There was no long-term follow-up of this patient.

Monochlorobenzene and p-dichlorobenzene have been associated with CNS depression in humans. Rozenbaum et al. (1947) examined 54 people, 28 of whom worked together in a factory where they had been exposed to monochlorobenzene vapors for one to two years. Headache, dizziness, somnolence, and dyspeptic disorders were reported by many of these workers. Examination revealed acroparesthesia (tingling, numbness, stiffness in extremities) in eight individuals, spastic contraction of finger muscles in nine individuals, hyperesthesia (excessive sensitiveness) of hands in four individuals, and spastic contraction of the gastrocnemius muscle in two individuals. The other 26 people, who had either short-term exposure to monochlorobenzene or were exposed to benzene and monochlorobenzene fumes, displayed no characteristic symptoms.

Wallgren (1953) reported on the examination of eight men who worked for one to seven months in a factory manufacturing mothproofing agents. These agents were produced from p-dichlorobenzene, which contained about 1% of an unidentified nitrogen-containing impurity and small amounts of o-dichlorobenzene. The workers developed neural disorders including intensified muscular reflexes, mild clonus of the ankle, and tremors of the fingers. The workers also experienced loss of appetite and hematopoietic changes.

Tarkhova (1965) studied the electroencephalographic patterns in 4 human subjects exposed by inhalation to 0.1, 0.2, or 0.3 mg/m^3 (0.02, 0.04 or 0.06 ppm) of monochlorobenzene. Changes were noted within minutes in the patterns of response to 10 nsec light flashes of 8-10 Hz and varying intensities by subjects exposed to the two higher concentrations. The lowest concentration appeared to produce no effect.

b. Animal Studies

In animal studies, nervous system effects have been shown following exposure to monochlorobenzene, o-dichlorobenzene, p-dichlorobenzene, and 1,2,4,5-tetrachlorobenzene.

Tarkhova (1965) exposed two groups of adult male white rats (15 per group) to monochlorobenzene by inhalation, at concentrations of either 0.1 or 1.0 mg/m^3 (0.02 or 0.2 ppm) continuously for 60 days. The higher-dose group showed increased cholinesterase activity in whole blood at 36 days and a reversal of the normal chronaxy ratio of antagonistic muscles at 39 days. Normally, the chronaxy (the relationship between a stimulus intensity and latency of response of the excitable tissue) of flexors is shorter than that of extensors, but exposure to certain toxic substances reverses that relationship. The group receiving 0.1 mg/m^3 of monochlorobenzene did not manifest these changes.

In studies by Varshavskaya (1967), groups of seven male albino rats were treated daily for nine months with monochlorobenzene or o-dichlorobenzene, in doses of 0.1, 0.01, or 0.001 mg/kg by stomach tube. With both monochlorobenzene and o-dichlorobenzene, the dose of 0.1 mg/kg produced deficits in the acquisition and performance of a conditioned response. Effects at the 0.01 mg/kg level were minimal.

In an investigation carried out on rats, Pislaru (1960) found that inhalation exposure to monochlorobenzene in concentrations of 0.1, 1.25, and 1.5 mg/l (22-326 ppm) produced chronaximetric modifications. As a result of exposure for 37 weeks at a concentration of 0.1 mg/l, chronaximetric inhibition was observed between weeks 7 to 14, after which the inhibition subsided. The author stated that chronaximetric changes preceded enzymatic changes (not specified) by 3 to 4 weeks.

Gabor and Raucher (1960) reported the effects on white rats of exposure to monochlorobenzene vapors at a concentration of 0.1 mg/l for three hours every other day for 37 weeks. Nervous system disturbances, indicated by inhibiting the chronaxy of the extensor tibialis, were observed from week 7 to 14; animals returned to normal by week 20.

Rabbits, rats, and guinea pigs exposed by inhalation to approximately 100 mg of p-dichlorobenzene for 20-30 minutes/day for 1-5 weeks exhibited tremors and twitches of the extremities, a "mark time" reflex, a loss of the righting reflex, a definite nystagmus, and rapid but labored respiration (Zupko and Edwards 1949).

Pike (1944) subjected several rabbits to as many as 62 exposures of eight-hour duration to p-dichlorobenzene vapors at a concentration of 4.6-4.8 mg/l (770-800 ppm) over a period of 83 days. Marked tremors and weakness were observed in all of the animals; only three survived 62 exposures. Lateral nystagmus and transitory edema of the cornea and optic nerve head were also observed. Repeated oral doses of p-dichlorobenzene at 1.0 g/kg by stomach tube five days/week produced marked intoxication, weakness, tremors, loss of weight, and death in some animals, but no eye effects were reported. Oral doses of 0.5 g/kg on the same schedule produced definite tremors and other signs of intoxication, but no deaths throughout one year of exposure.

Hollingsworth et al. (1956) found symptoms of central nervous system effects including tremors and weakness in rats, guinea pigs, and rabbits exposed to 4.8 mg/l (798 ppm) of p-dichlorobenzene vapors, 8 hours/day, 5 days/week, for up to 69 exposures. No neurological effects were reported at 341 ppm or less in 6 months.

Fomenko (1965) reported central nervous system effects in rats treated with 1,2,4,5-tetrachlorobenzene. The rats were treated after a conditioned response to a stimulus had been established. A dose-dependent alteration of conditioned responses was observed in rats intubated with 0.005 mg/kg/day and 0.05 mg/kg/day for eight months. The conditioned responses became slower and decreased in magnitude. More responses during extinction and a longer time to restoration of the response were also noted. At 0.05 mg/kg, the response was not restored, i.e., was completely inhibited.

2. Decision

Evidence cited in this document indicates that the chlorinated benzene congeners are hazardous to the integrity of neurobehavioral functioning in humans and animals. Signs and symptoms of adverse effects on the nervous system in various species, including humans, rats, rabbits, and guinea pigs have been associated with exposure to four of the congeners (monochlorobenzene, _o_-dichlorobenzene, _p_-dichlorobenzene, and 1,2,4,5-tetrachlorobenzene).

The substances are non-specific central nervous system (CNS) depressants. While the chlorinated benzenes vary in potency, the neural effects produced in humans and animals by the various compounds are similar. Effects observed in humans include somnolence and loss of consciousness, dizziness, headache, loss of appetite, clonus, disturbance of innate reflexes, tremors, and spastic contractions; the last three effects occurred in animals as well. Also found in animals were disturbance of conditioned reflexes, changes in the chronaxy ratio of antagonistic muscles, nystagmus, and weakness.

For the chlorobenzene compounds that have been tested for neurotoxic and behavioral effects, the dose-response characterization is incomplete. Moreover, available observational data are poorly quantified, subjective, and therefore, relatively insensitive. Subchronic studies of electrophysiological functions are inadequately detailed. Better data are needed for a more complete characterization and assessment of the hazard from exposure to the chlorinated benzenes.

Russian reports suggest that a no-effect level for neurobehavioral effects of the chlorobenzene congeners may be very low. Adverse effects have been reported in continuous inhalation experiments on rats using as little as 0.2 ppm of monochlorobenzene in air. These studies suggest that functional effects may ensue from exposure to chlorinated benzenes at concentrations lower than those that produce overt pathology.

The Agency believes that testing of the chlorinated benzenes for their neurotoxicity will provide data relevant to determining whether exposure to these substances presents an unreasonable risk to human health from neurotoxic and behavioral effects and is therefore considering what testing might be undertaken with respect to these effects. EPA is not proposing specific neurotoxicity or behavioral effects testing at this time because the Agency has not yet developed test standards for such testing.

3. Testing Under Consideration

The following discussion sets forth the Agency's current views on testing chlorinated benzenes for neurotoxicity and behavioral effects. EPA is proposing that such tests include both acute and subchronic (repeated exposure for 90 days or longer) tests on rodents using locomotor activity, a functional observational battery, a neurophysiologic test of chronaxy, and an appropriate electrodiagnostic test. Histopathology of the nervous system of subchronic test animals is also recommended. This examination should include: longitudinal and cross sections of the spinal cord, i.e., thoracic and lumbar regions; cross sections of the forebrain, midbrain, and brainstem; and representative sections of the sciatic nerve. Tissue should be fixed *in situ* with formaldehyde or glutaraldehyde and paraformaldehyde.

Tests of locomotor activity have been widely used in screening drugs and have been proposed as screening tests for environmental chemicals. A recent survey by Reiter and MacPhail (1979) of locomotor activity measures discusses some of the problems involved in generating comparable data from different types of devices as well as the influence of other important variables. They conclude that in general, when combined with observational measurements of other central nervous system functions, automated activity devices provide more reliable and better quantified measures of locomotor activity.

Observational assessment by means of screening tests that measure objective physiologic signs, unconditioned reflexes, elicited responses, and operants are essential for detecting the spectrum of an agent's effects and providing a basis for determining the agent's functional anatomical targets. Tilson and Cabe (1978) and Tilson, Mitchell, and Cabe (1979) present useful examples of a screening battery and discuss some factors important to development of screening batteries.

The neurobehavioral functions assessed in the literature on chlorinated benzenes by means other than observation are acquisition of conditioned responses, chronaxy measurements of nerves or muscles, and electroencephalography. EPA is proposing that subchronic studies of the effects of chlorobenzenes include measurement of chronaxy and some other neural function. Among such functional tests, conduction velocity of a mixed large and small diameter fiber population (see, for example, Glatt et al. 1979) is a well-known parameter for evaluating nerve damage. However, other tests such as frequent impulse series transmission (e.g. Tackmann et al. 1975) or other electrodiagnostic procedures should be considered. The Agency is interested in comments on the suggested neurotoxicity tests, particularly on the adequacy of rodents as the proposed test species and on the appropriate duration of exposure for obtaining data for risk assessment.

D. Reproductive Effects

1. Evaluation of Pertinent Studies

a. Human Case Reports

No reports of reproductive effects in humans exposed to chlorinated benzenes are known to EPA.

b. Animal Studies

The Monsanto Co. (1978g) reported gonadal effects in a study in which dogs were exposed to monochlorobenzene vapor at 0, 0.76, 1.47, and 2.00 mg/L for 6 hours/day, 5 days/week for a total of 62 exposures. In the high dose group, two of four male dogs developed bilateral atrophy of epithelial tissue in the seminiferous tubules. These effects are consistent with effects found in an earlier subchronic study in which groups of four male and four female dogs were dosed with 0.025, 0.050, and 0.250 mg/kg/day orally for 13 weeks (Monsanto 1967a). In this earlier study, decreased spermatogenesis was seen in three of the four dogs in the high dose group. Tubular atrophy and epithelial degeneration were also seen in this group.

In the Monsanto (1978g) study, rats exposed to the same concentrations and test conditions as the dogs showed less definite gonadal responses; female rats exposed to 2.0 mg/l of monochlorobenzene exhibited a significantly higher gonad to body weight ratio than untreated females ($p < 0.01$).

c. Other Indicators for Reproductive Effects

Evidence for the potential of chlorinated benzenes to affect reproduction can be inferred from the effects of hexachlorobenzene on reproduction. This compound is related to chlorobenzenes structurally, and in producing some effects such as hepatotoxicity it is similar to the other chemicals of the group. Hexachlorobenzene was given orally to five female monkeys for 60 days (Iatropoulos et al. 1976). Three monkeys received 8, 32, and 64 mg/kg/day, respectively, and two others received 128 mg/kg of hexachlorobenzene. One control monkey received vehicle only. Fourteen monkeys of similar weight and age were also used as controls but did not receive the vehicle. At the lowest dose given, changes occurred in the germinal epithelium of the ovaries. Multiple follicular cysts were observed in all hexachlorobenzene-treated animals except in the animal treated with 32 mg/kg. The ovarian cortices of monkeys from all treated groups showed varying degrees of degeneration. The number of primary follicles was markedly reduced by 17, 17, 71, and 80% for the monkeys given 8, 32, 64, or 128 mg/kg, respectively. The changes involved all ovarian elements including primary follicles, germinal epithelium, and stroma; they were morphologically similar to postmenopausal changes. Apparently, the corpora lutea were not receptive to gonadotropin stimulation or were deficient in steroidogenesis, or the uterus failed to respond to luteal transition, since the endometrium of all dosed animals remained in the follicular phase. Minor liver changes as well as the changes in the ovarian pathology and function occurred in the low dose animal. Increased ovarian and liver pathology occurred at the higher doses.

A four generation reproduction study in the rat in which groups of 20 female and 10 male rats per group were given, respectively, 0, 10, 20, 40, 80, 160, 320, and 640 ppm of hexachlorobenzene in the feed demonstrated several reproductive effects (Grant et al. 1977). The two highest dietary concentrations caused 10% and 50% death respectively, in the parental generation. Viability indices of the F_1 generation were severely decreased in groups fed 160 ppm or more in the diet. The lactation index was reduced in the F_3 generation of the 80 ppm group, the highest dose group for which there was an F_3 generation. Fertility was affected in the two highest dose groups. The relative liver weights and aniline hydroxylase activities were increased in weanlings from dams fed 40 ppm of hexachlorobenzene, the only dose for which the enzyme activity was reported.

The levels of hexachlorobenzene in the plasma, brain, kidney, liver, and body fat of 21 day old pups from dams fed 0, 10, 20, 40, 80, and 160 ppm of hexachlorobenzene were determined (Grant et al 1977). The highest concentration of hexachlorobenzene was found in the body fat. The hexachlorobenzene content of the pups increased with the dietary content of the compound fed to the dams. The high concentrations observed in the pups in relation to the dietary intake of the dam suggest a high rate of excretion of HCB via the milk. The residue data also indicate that the pups can tolerate relatively high body burdens without showing any adverse effects on the various parameters measured in this study.

2. Decision

Studies in monkeys indicate that hexachlorobenzene causes dose-related ovarian effects after 60 days' exposure to a dose as low as 8 mg/kg/day. A multigeneration study in rats dosed with hexachlorobenzene reported fertility problems in animals given 320 or 640 ppm in feed, and decreased fetal viability in F_3 generation animals exposed to as little as 80 ppm. Decreased spermatogenesis and atrophy of seminiferous tubule tissue has been noted in dogs receiving monochlorobenzene. The totality of these results raises concern that other chlorinated benzenes may elicit similar responses and that consequently they should be tested for reproductive effects.

3. Proposed Testing

EPA is proposing testing of the chlorinated benzenes except 1,2,4-trichlorobenzene for reproductive effects. Since a reproductive effects study on 1,2,4-trichlorobenzene, requested by the EPA Office of Drinking Water, is nearing completion at EPA's Health Effects Research Laboratory in Research Triangle Park, North Carolina, further testing of this compound appears unnecessary unless evaluation of the final report reveals deficiencies in the EPA study. Standards for development of test data on reproductive effects have been proposed (USEPA 1979d). Adherence to these standards should produce data necessary to define the hazard to reproduction from exposure to chlorinated benzenes.

E. Teratogenic Effects

1. Review of Pertinent Studies

a. Morphological Teratogenicity

i. Human Case Reports

No epidemiology studies or human case reports were found which indicate that exposure to chlorinated benzenes is associated with teratogenic effects.

ii. Animal Studies

Investigators have demonstrated fetal anomalies and malformations resulting from the exposure of pregnant rats and mice to pentachlorobenzene and hexachlorobenzene.

Khera and Villeneuve (1975) reported the testing of pentachlorobenzene for teratogenic potential. Groups of pregnant Wistar rats were given pentachlorobenzene dissolved in corn oil at doses of 0, 50, 100, or 200 mg/kg/day by gavage on days 6-15 of gestation. No signs of toxicity were reported in dams throughout the test. There were no significant differences in the average number of live fetuses/litter and in the ratio of fetal deaths to total implants. There was a statistically significant increase in the incidence of unilateral and bilateral extra rib formation in fetuses in all test groups. Unilateral extra ribs occurred in 1.6% of the controls while pentachlorobenzene-treated animals showed 14%, 8%, and 17% incidence, at 50, 100, and 200 mg/kg, respectively. The incidence of bilateral extra ribs was 1.6% for controls, 8% at 50 mg/kg, 9% at 100 mg/kg, and 46% at 200 mg/kg. In addition, the number of litters in which one or more fetuses had rib anomalies (14th and 15th combined) were 3 of 19 for controls, 14 of 19 at 50 mg/kg, 11 of 19 at 100 mg/kg, and 15 of 19 at 200 mg/kg. The increased incidences were significant ($p<0.001$) at all dose levels. Other workers treated pregnant CD-1 mice with 50 or 100 mg/kg of purified pentachlorobenzene by gastric intubation in corn oil on days 6 to 15 of gestation (Courtney et al. 1977). The maternal liver to body weight ratio was significantly increased in both dosage groups. Although the fetal weight was reduced in both dose groups and one cleft palate occurred (10 litters with 11± 4 live/litter) in the 50 mg/kg dose group, no dose-related malformations occurred.

A screening-type teratology study on 1,2,4-trichlorobenzene, requested by the EPA Office of Drinking Water, is nearing completion at EPA's Health Effects Research Laboratory in Research Triangle Park, North Carolina. When a report on the results of this study becomes available, and if the screen becomes validated as an indicator of teratogenic potential, EPA will take the test results into consideration in determining the need to include 1,2,4-trichlorobenzene in a final teratology test rule for the chlorinated benzenes.

The chlorobenzene producers are reportedly (Dow 1978b) planning jointly sponsored teratology studies on monochlorobenzene, *o*-dichlorobenzene and *p*-dichlorobenzene. The National Toxicology Program nominated *p*-dichlorobenzene for teratogenicity testing, but it has not yet made a final decision to test the chemical (USDHEW 1979).

iii. Other Indicators of Teratogenic Potential

(1) Structural Relationship with Hexachlorobenzene

Hexachlorobenzene was studied for teratogenic potential in mice. Ten pregnant CD-1 mice were given hexachlorobezene (100 mg/kg by oral intubation) on days 7 through 16 of gestation. Small kidneys, renal agenesis, enlarged renal pelvis, club foot, and cleft palate were observed ($p<0.05$) (Courtney et al. 1976). Although only one dose group and ten animals were used, the study indicates that hexachlorobenzene is teratogenic in mice.

In another study, hexachlorobenzene was administered orally to rats at a dose of 0, 10, 20, 40, 60, 80, or 120 mg/kg on each of days 6-9, 10-13, 6-16, and 6-21 of gestation (Khera 1974). A significant increase in the incidence of 14th rib (uni- and bilateral) in hexachlorobenzene-treated groups compared with that in the relevant control group was observed in the groups treated on days 10-13, 6-16, and 6-21 of gestation. The incidence increased as a function of dose and length of treatment. Sternal defects and retarded ossification were significantly more frequent in the group treated on days 6-21 and were dose-related. The report indicates the rib anomalies were not confirmed in subsequent trials at doses up to 80 mg/kg. No test results or further discussion were presented.

(2) Potential for Placental Transfer

It is likely that the chlorinated benzenes can pass from the maternal circulation into the fetal unit. Non-ionized chemicals with high lipid solubility readily cross the placental membranes and gain access to the developing embryo (Fingl and Woodbury 1970, Nishimura and Tanimura 1976). Monochlorobenzene has an octanol/water partition coefficient of log $P_{oct} = 2.84$ and those of higher chlorinated benzenes range from 3.38 to an upper limit expected to fall between the 4.1 observed for a trichlorobenzene and the 5.8 found for hexachlorobenzene (Table 1, Section I). The relatively low molecular weights and the lipid solubility of the chlorinated benzenes indicate a potential for rapid diffusion across the placenta. Furthermore, because chlorobenzenes are non-specific CNS depressants in adults and therefore must cross the blood-brain barrier, the chlorinated benzenes or their toxic metabolites can probably cross the placenta as well. Khera (1974) pointed out in the introduction of his paper that the appearance of hexachlorobenzene in the body fat and milk of exposed humans has been reported by several other investigators. Thus the exposure of pregnant females to chlorinated benzenes may result in exposure of the embryo, fetus, and neonate.

(3) Teratogenicity of Chlorinated Benzene Metabolites

The fact that chlorinated benzenes are metabolized to chlorinated phenols (Parke and Williams 1955, Kohli et al. 1976; see Section III.B.1.c.(1), Figures 2-6) is of concern because one chlorinated phenol has shown embryotoxicity and another caused minor developmental effects in animal tests.

Pentachlorophenol was given to rats at dose levels of 5, 15, 30, and 50 mg/kg on days 6-15 inclusive of gestation; the 30 mg/kg dose caused statistically significant resorptions while the 5 mg/kg dose caused a statistically significant increase in delayed skull ossification (Schwetz et al. 1974a). At doses above 5 mg/kg, minor signs of embryotoxicity were observed such as delayed skull ossification, lumbar spurs, vertebral and sternal anomalies, and subcutaneous edema, each statistically significant.

2,3,4,6-Tetrachlorophenol has been tested for teratogenic potential at doses of 10 or 30 mg/kg on days 6-15 of pregnancy (Schwetz et al. 1974b). A statistically significant increase in subcutaneous edema was observed in the 10 mg/kg dose group but it did not appear to be dose-related. An increase in delayed skull ossification observed at the 30 mg/kg dose was not statistically significant. Some of the minor anomalies observed in this study also occurred with hexachlorobenzene, pentachlorobenzene and pentachlorophenol.

b. Behavioral Teratogenicity

Additional concern for the teratogenic potential of chlorobenzenes is based on their documented neurotoxicity. Acute and repeated exposure to all of the tested chlorinated benzenes (in animals: monochlorobenzene, ortho- and para-dichloro- and 1,2,4,5-tetrachlorobenzene; in humans: monochlorobenzene and para-dichlorobenzene) have produced adverse effects on the central nervous system (CNS) (see Section III.C.). The central nervous system appears to be especially susceptible to toxic insult during its development (Buelke-Sam and Kimmel 1979). The period during which the CNS develops is an extended one and vulnerability to toxic insult continues into the post-natal period. The possibility of fetal exposure to neurotoxicants such as the chlorinated benzenes warrants their evaluation as teratogens. Evidence has been presented that suggests that both structural and behavioral deficits in adult and developing systems are associated with exposure to other non-specific CNS depressant chemicals (van Stee 1976). Few purely behavioral teratogens are known at this time, but psychotropic drugs which have little or no structural teratogenic potential have been identified as behavioral teratogens (Vorhees et al. 1979a). Chlorinated benzenes therefore may have the potential for causing neurobehavioral problems in newborns, even if no classical terata are produced.

2. Decision

Several factors indicate that the chlorinated benzenes may have teratogenic potential. They are related structurally to hexachlorobenzene, which is teratogenic in mice. Pentachlorobenzene causes rib abnormalities in rats similar to those caused by hexachlorobenzene. Although pentachlorobenzene does not cause overt malformation in the rat, the rib abnormalities are dose-related. EPA considers the appearance of extra ribs in the rat to be suggestive but not conclusive evidence for teratogenic potential, and is interested in receiving comment on this point. Certain phenolic metabolites of the chlorinated benzenes are also known to cause embryo- and fetotoxic responses in the rat. The chlorinated benzenes have been associated with adverse central nervous system effects in humans and animals; these substances and some of their toxic metabolites are likely to cross the placenta and thus may pose a hazard to the developing embryo or fetus for both morphological and behavioral teratogenicity. The EPA is proposing teratogenicity testing on the chlorinated benzenes except pentachlorobenzene, which has been adequately tested and needs no further studies to evaluate its teratogenic potential.

3. Proposed Testing: Morphological Teratogenicity

Standards for development of data for teratogenic health effects have been proposed (USEPA 1979c). Adherence to these standards should produce data necessary to define the structural teratological hazard from exposure to chlorinated benzenes.

4. Testing Under Consideration: Behavioral Teratogenicity

EPA is considering requiring behavioral teratogenicity testing of chlorinated benzenes. The Agency believes that behavioral teratogenicity testing should include evaluation of behavioral and neurological development in the offspring of pregnant animals exposed to environmental contaminants and industrial agents (Vorhees et al. 1979b). In addition to routine parameters of physical development that may reflect toxicity, such as body weight, the proposed testing should include specific tests to assess in offspring the known effects of chlorinated benzenes in adults. As non-specific CNS depressants, the chlorinated benzenes cause narcosis, reflex changes, and other neurological motor signs as well as changes in food intake and body weight (see Section III.C, Neurotoxic Effects). Screening batteries specifically designed for examining these behaviors in developing organisms should include measures shown to be related to intoxication by chlorinated benzenes. Neuropathology should also be included. The Agency is interested in comments on the suggested behavioral teratogenicity tests.

F. Mutagenic Effects

1. Evaluation of Pertinent Studies

Chlorinated benzenes have been tested for mutagenic activity in several systems including bacteria, yeast, mammalian cells in culture, and plants. The results of these studies are summarized below.

a. Gene Mutation Studies

Keskinova (1968) reported that the frequency of induced mutations in *Streptomyces antibioticus* (in Russian nomenclature, *Actinomyces antibioticus)* 400 after exposure to gaseous monochlorobenzene was 1400 times the mutation frequency seen in negative control cultures. Liver activation was not employed. Monochlorobenzene induced point mutations in this system. Although it appears that monochlorobenzene is a direct-acting mutagen (i.e., does not require metabolic activation), it is possible that the organism used in this assay possesses the enzymes necessary to metabolize the compound to a mutagenic form.

Monochlorobenzene and *o*-, *m*-, and *p*-dichlorobenzene were tested at a concentration of 200 ug/ml to determine their ability to induce reverse mutation in methionine and pyridoxine auxotrophs of *Aspergillus nidulans* (Prasad 1970). Liver enzyme activation was not employed. Monochlorobenzene was nonmutagenic in this assay. The dichlorobenzenes appeared to have mutagenic activity, however. This activity increased in the order o-dichlorobenzene, m-dichlorobenzene, p-dichlorobenzene, with p-dichlorobenzene being approximately twice as active as o-dichlorobenzene. As stated above for monochlorobenzene, the dichlorobenzenes may be direct-acting mutagens or may be activated by enzymes present in Aspergillus. Although the dichlorobenzenes appear to be active in this study, there are several points that should be taken into consideration. The spontaneous mutation rate was much lower than normal for Aspergillus; the dichlorobenzenes were tested at only one concentration, 200 ug/ml; and the number of plates per experiment was not given although it was stated that duplicate experiments were done. To be considered fully valid, a test should be performed with 15 plates per point for at least three dose levels. Nevertheless, the EPA considers this study to be an indication of the activity of the dichlorobenzenes in Aspergillus and feels that this is an appropriate system for initial testing of the chlorinated benzenes for gene mutational activity (see Appendix B).

Chlorinated benzenes have been reported to be inactive in bacterial tests for mutagenicity using histidine auxotrophs of Salmonella typhimurium as indicators of mutagenic potential (E. I. duPont 1977, Merck 1978, Monsanto 1976a, 1976b, Monsanto 1977a, 1977b, 1977c, Monsanto 1978d, 1978e, 1978f, Simmon et al. 1979).

Monochlorobenzene was tested in plate incorpation assays with Salmonella strains TA 1535, TA 1537, TA 1538, TA 92, TA 98, and TA 100 both with and without liver enzyme activation over a range of 0.01 ul - 5.0 ul per plate (Monsanto 1976a, 1976b, Simmon et al. 1979) and 100 ml per plate (Merck 1978), and at concentrations of 150-3000 ug/plate (E. I. duPont 1977); the compound was negative at all levels.

o-Dichlorobenzene was tested in plate incorporation assays with Salmonella strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100, both with and without liver enzyme activation, at concentrations of 0.6-1300 ug/plate (Monsanto 1977a, 1978e) and 0.05-1.0 ul/plate (Simmon et al. 1979). It was found to be nonmutagenic in these assays.

m-Dichlorobenzene was tested both with and without liver enzyme activation in plate incorporation assays with *Salmonella* strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 (Monsanto 1977b, 1978d, Simmon et al. 1979) at concentrations of 0.6-1300 ug per plate. It was found to be nonmutagenic at all concentrations tested.

p-Dichlorobenzene at concentrations of 0.5-1000 ug/plate was found to be nonmutagenic for *Salmonella* strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 when tested both with and without rat liver enzyme activation (Monsanto 1978f, Simmon et al. 1979) in a plate incorporation assay.

Schoeny et al. (1979) reported that 1,2,4-trichlorobenzene at concentrations of 102-2914 ug/plate was nonmutagenic for *Salmonella* strains TA 1535, TA 1537, TA 98, and TA 100 when tested both with and without liver enzyme activation.

The National Institute of Environmental Health Sciences has scheduled all of the chlorinated benzenes for mutagenicity testing under the National Toxicology Program. The compounds will be tested in the *Salmonella*/microsomal assay. Tests on the six di- and trichlorobenzenes are scheduled for completion in fiscal year 1980; testing of the remaining five compounds will begin before the end of fiscal year 1980 (USDHEW 1979).

Monochlorobenzene and *o*-, *m*-, and *p*-dichlorobenzene tested at the same concentrations as those reported above for *Salmonella* were found to be nonmutagenic for *E. coli* WP2 (Simmon et al. 1979) when tested both with and without liver enzyme activation.

Monochlorobenzene was tested for its ability to induce specific locus forward mutations in the mouse lymphoma L5178Y TK$^+$ /$^-$ assay system (Monsanto 1976c). The test was performed both with and without mouse liver enzyme activation. Monochlorobenzene was tested at concentrations of 0.001 ul/ml to 0.1 ul/ml without activation and at concentrations of 0.0001 ul to 0.01

ul/ml with activation. Monochlorobenzene was found to be nonmutagenic for L5178Y cells in these studies. *o*-, *m*- and *p*-Dichlorobenzene have not been tested in this system.

b. Chromosomal Aberration Studies

(1) Mitotic Gene Conversion and Recombination in Yeast

Monochlorobenzene and *o*-, *m*-, and *p*-dichlorobenzene were tested for their ability to induce reciprocal recombination in *Saccharomyces cerevisiae* strain D3 (Simmon et al. 1979). Monochlorobenzene at concentrations of 0.05% and 6% in the presence of an S-9 mix produced a reproducible increase in the number of recombinant cells per 10^5 survivors over that seen in negative control cultures (Simmon et al. 1979). Little or no recombinogenic activity was observed in the *S. Cerevisiae* D3 assays with *o*-dichlorobenzene. Although enzyme activation enhanced the recombinogenic activity of *m*-dichlorobenzene, it was not necessary. *m*-Dichlorobenzene at concentrations of 0.001% - 0.025% was inactive in this system both with and without metabolic activation. Results with *p*-dichlorobenzene were inconsistent and not reproducible. In three experiments, recombination was increased, but survival data were inconsistent. In two subsequent experiments, survival data were more consistent, but no recombinogenic activity was noted. Monochlorobenzene at concentrations of 0.01-5.0 ul/plate did not induce mitotic conversion at the trp 5 locus of *S. cerevisiae* strain D4 when tested with and without liver enzyme activation (Monsanto 1976a,b).

(2) Tests for Chromosomal Effects in Plants

Chromosomal effects can be measured in microorganisms, plants, mammalian cells in culture, and whole animals. Although the events measured in plants are similar to those in mammalian cells, their relevance to man is sometimes questioned. However, an examination of the literature comparing chromosomal effects demonstrated in plants with those in mammalian cells in culture shows an excellent degree of correlation between the two systems (Flamm 1977).

Ostergren and Levan, in a preliminary report (1943), indicated that monochlorobenzene, o- and m-chlorobenzene, 1,2,3-trichlorobenzene, and hexachlorobenzene display C-mitotic activity toward plant cells of *Allium cepa*. The authors proposed that the C-mitotic properties of organic compounds are not due to their chemical properties (e.g. reactivity) but to their physical properties (e.g. water solubility).

p-Dichlorobenzene has been reported to cause chromosome breaks in a variety of plant systems (Sharma and Bhattacharyya 1956, Sharma and Sarkar 1957, Srivastava 1966, Gupta 1972). Although these studies are difficult to evaluate because neither control data nor quantitative dose-response data were presented, it is apparent that p-dichlorobenzene does have an effect on chromosomal structure.

c. DNA Repair Studies

The relative toxicity of monochlorobenzene and o-, m-, and p-dichlorobenzene was assessed in repair-proficient and repair-deficient strains of *E. coli* (pol A^+/pol A^-) and *Bacillus subtilis* (Rec^+/Rec^-) (Simmon et al. 1979). o- and m-Dichlorobenzene produced differential cell kill of repair proficient and deficient strains of *E. coli*.

Monochlorobenzene at concentrations of 10 and 20 ul/plate was equally toxic to both the pol A^+ and pol A^- strains of *E. coli* and to rec^+ and rec^- strains of *B. subtilis*.

o- and m-Dichlorobenzene at 20 ul/plate were more toxic to *E. coli* strain p3478 (pol A^-) then they were to *E. coli* strain W3110 (pol A^+). o-Dichlorobenzene and m-dichlorobenzene were each equally toxic to both the rec^- and rec^+ strains of *B. subtilis*.

p-Dichlorobenzene at concentrations of 1 and 5 ul/plate had no effect upon either the *E. coli* or the *B. subtilis* strains. It may be that p-dichlorobenzene is nontoxic at the concentrations tested or that it failed to diffuse away from the filter paper disc into the medium to come in contact with the bacteria.

2. Decision

The chlorinated benzenes have been reported to produce gene mutation in some bacteria and fungi, mitotic recombination in yeast, DNA damage in bacteria, and C-mitosis and chromosome breaks and abnormalities in plants.

Monochlorobenzene, *o*-, *m*-, and *p*-dichlorobenzene and 1,2,3-trichlorobenzene have been tested and found positive in one or more systems designed to assess these effects. The first four of these compounds have also been tested in the *E. coli* WP2 and *Salmonella*/microsomal assays with negative results.

Positive results in a test system are clear indications of mutagenicity. Negative results in such systems are a bit ambiguous. A negative result in a back mutation test, for example, may mean that the chemical was non-mutagenic under the test conditions, i.e., that it is mutagenic but the activity could not be expressed. The agent may not be able to interact with DNA at the specified locus, or mutations may be occurring at sites in the DNA which are not detected in the test system. Inactivity in any test system may mean that the conditions of the test including pH, cofactors, and time of exposure were not optimal for the test agent or that the metabolic activation system was inappropriate for the chemical under test. The significance of negative results in either bacterial or mammalian cell culture assays must be assessed in relation to results in other assays and viewed as part of a continuum rather than as absolute reflections of mutagenic potential.

The activity of monochlorobenzene in the *S. antibioticus* 400 system and the activity of the dichlorobenzenes in *A. nidulans* show that these chlorinated benzenes do cause point mutations. The chlorobenzenes are also genetically active in yeast where they induce mitotic recombination in *S. cerevisiae*. Mitotic recombination can result in the expression of a recessive homozygous condition which would not be expressed in the heterozygous state. There is evidence that mitotic recombination occurs in all eukaryotes, including mammals (Flamm 1977). Although the significance of mitotic recombination in the human population is unknown, the ability of the chlorobenzenes to induce recombination indicates that they are potentially genetically active agents. This potential is further emphasized by the ability of these agents to interact with bacterial and plant DNA.

In summary, the chlorinated benzenes have mutagenic activity. Given the weight of the evidence, EPA considers the chlorinated benzenes to have the potential to mutate the human genome, which may pose a genetic risk to the population. Further testing of these agents is needed to define the degree of hazard that these agents represent to humans. However, as explained below, EPA is not proposing such testing at this time.

3. Testing to be Sponsored by EPA

EPA believes that mutagenic risk from exposure to chlorinated benzenes can most reasonably be determined by performing a sequence of tests for both gene mutation and chromosomal aberration; the testing sequence under development by EPA is described in Appendix B. In such schemes, the performance of certain tests is triggered by positive or negative results from previous tests. At this time, EPA is not proposing test requirements for the mutagenicity sequence because the Agency has not yet defined the criteria for determining whether the results of each test are positive or negative. Such criteria are important if the Agency is to establish a sequential testing process in one rulemaking under Section 4. In addition, EPA has not yet developed test standards to be followed for the DNA alkylation tests in the gene mutation sequence, which is the uppermost test in the proposed testing sequence. Nevertheless, testing of chlorobenzenes for their mutagenic effects should not be delayed solely because EPA cannot yet put in place all elements necessary for the testing sequence. Since the tests are relatively inexpensive, EPA plans to sponsor all tests in the sequences except the two DNA alkylation tests for gene mutation and the heritable translocation assay for chromosomal aberration. On the basis of its test results, EPA will decide whether to propose that the final tests of each sequence be performed.

EPA wishes to avoid duplicative testing where possible. Therefore the Agency will coordinate its planned mutagenicity tests on chlorinated benzenes with any such testing planned or contemplated by the National Toxicology Program, which, as described above under "Gene Mutation Studies," has scheduled some mutagenicity tests on chlorinated benzenes.

G. Oncogenic Effects

1. Evaluation of Pertinent Studies

No tests for the oncogenicity of the chlorobenzenes have been identified in the published literature. Although toxicity studies by Hollingsworth et al. (1956, 1958) on *o*- and *p*-dichlorobenzene were termed carcinogenicity testing by Ware and West (1977), these investigations were conducted for 5 to 7 months and therefore are too short to be considered as oncogenicity tests. There are, however, human case reports of leukemia associated with exposure to the chlorobenzenes.

a. Human Case Reports

Four cases of human leukemia have been associated with exposure to the chlorinated benzenes (Girard et al. 1969, Tolot et al. 1969).

The first case (Girard et al. 1969) involved a man hospitalized for chronic lymphoid leukemia, after having worked with a solvent containing 80 percent *o*-dichlorobenzene, 15 percent *p*-dichlorobenzene and 2 percent *m*-dichlorobenzene for 10 years. This same solvent was implicated in the development of acute myeloblastic leukemia in a 55-year-old woman who used it for an unspecified period to clean spots from her family's clothes. In another case (Girard et al. 1969), a 15-year-old girl was hospitalized with acute myeloblastic leukemia and died 10 months later of peripheral leukoblastosis. She had habitually removed dirt and grease stains from the clothes she was wearing with a product containing 37 percent *o*-dichlorobenzene. No further details of these incidents were given.

Tolot et al. (1969) presented the case of a 40-year-old man who had been exposed to o-dichlorobenzene for 22 years in the preparation of dyestuffs. The subject exhibited purpura, intense anemia, marked hepatomegaly, and discrete splenic enlargement. The man died four months after the case was diagnosed as myelocytic (myeloblastic) leukemia.

The absence of controlled conditions at the time of exposure in all these case reports makes it difficult to determine cause and effect. Thus, the case reports discussed provide only inconclusive evidence of the leukemogenic potential of the chlorinated benzenes.

b. Animal Studies

The Imperial Chemical Industries long-term inhalation study in rats on p-dichlorobenzene referred to in Section III.B.1.b.(2) may provide information on oncogenicity. Any such information that becomes available will be included in EPA's evaluation of the chlorinated benzenes.

Three of the chlorinated benzenes, monochlorobenzene, o-dichlorobenzene, and p-dichlorobenzene are being tested in long-term bioassays directed by the National Cancer Institute (NCI). Testing of monochlorobenzene began in January of 1979 and is scheduled to be completed in February 1981. o-Dichlorobenzene testing began in March 1979 and is scheduled for completion in February 1981. Testing of p-dichlorobenzene will begin in June of 1980 and is scheduled for completion in June of 1982.

c. Other Indicators of Oncogenic Potential

The following sections discuss information that is often suggestive of oncogenic potential but which is usually inadequate evidence by itself to address the oncogenic hazard of a substance.

(1) Mutagenic Activity

The concept that neoplasms arise from mutations in somatic cells was originally postulated by Boveri in 1914 to account for the unlimited variety of tumor types and the fact that, on cell division, the daughter cells maintain their neoplastic properties (Boveri 1929, Chu et al. 1977, Trosko and Chang 1978). Oncogens and mutagens have two properties in common: 1) the ability to transmit newly induced properties to their daughter cells and 2) the ability to convert normal cells into irreversibly changed cells (Suss et al. 1973). Although the mutation theory of oncogenesis is still waiting for unequivocal experimental proof, the theory has recently gained more attention because of three important findings. First, in the 1960's, the Millers at the University of Wisconsin discovered that a majority of oncogens required metabolic activation (Miller and Miller 1974, Miller 1978, Miller 1979); second, *in vitro* metabolic activation systems which could be incorporated into mutagenicity assay systems were developed (Malling and Chu 1974); and third, comparison of the ultimate reactive metabolites of structurally diverse oncogens and mutagens revealed that the common denominator of these substances is their electrophilicity, (i.e., they are compounds that are able to react with electron-rich sites, or nucleophiles, in cellular nucleic acids and proteins) (Bartsch 1976, Miller 1979). These three findings have now been verified by a host of experimental data which show that oncogens can induce different types of mutations including gene mutations (both base-pair substitution and frame-shift alterations), chromosomal aberrations, and non-disjunctions. The oncogenic potential of a chemical has been correlated with its ability to interact with and modify DNA (Rosenkranz and Poirier 1979). These considerations have resulted in the general acceptance of mutagenicity tests as indicators of oncogenic potential (Miller 1978).

The chlorinated benzenes have been evaluated for mutagenic activity in a number of test systems including bacteria, fungi, yeast, mammalian cells in culture, and plants. The results of these studies indicate that several of the chlorinated benzenes possess mutagenic potential. In addition, the chlorinated benzenes have been shown to produce DNA damage in bacterial systems and chromosomal damage in plants. These studies are discussed in detail in the Mutagenicity Section of this document (Section III. F). Although the exact relationship between a chemical's mutagenic activity in these systems and its oncogencity is unknown, EPA must consider these positive mutagenicity test results suggestive evidence of the oncogenic potential of the chlorinated benzenes.

(2) Structural Relationships

Known chemical oncogens comprise a stsructurally diverse group of substances. (Miller and Miller 1974, Miller 1979). However, many chemical oncogens can be divided among a smaller number of structural classes, and a structural relationship to a known oncogen or class of oncogens is a reasonable basis for suspecting an untested chemical of oncogenic activity (Arcos 1978).

The chlorinated benzenes are structurally similar to two recognized oncogens: benzene, which has been shown to produce tumors in Sprague-Dawley rats (Maltoni and Scarnato 1979) and which has been implicated as a leukemogenic agent in humans (Aksoy et al. 1972, 1976, Tareeff et al. 1963), and hexachlorobenzene, which has been shown to produce tumors in syrian golden hamsters (Cabral et al. 1977) and in outbred Swiss mice (Cabral et al. 1979).

(a) Benzene

There have been over 100 case reports, a number of epidemiologic studies, and an animal study implicating benzene as an oncogen (Tareeff et al. 1963, Aksoy et al. 1972, 1976, USEPA 1978a, Maltoni and Scarnato 1979). It has been established that benzene is toxic to the human hematopoietic system and may induce blood and bone marrow changes that may in turn lead to the appearance of leukemia in man (USEPA 1978a). Most individuals and scientific groups have accepted a causative role for benzene in the development of human acute myelogenous (myeloblastic) leukemia (USEPA 1978a).

The recent study by Maltoni and Scarnato (1979) reveals the oncogenic potential of benzene in laboratory animals. In this study, groups of 60 and 70 Sprague-Dawley rats (13 weeks old at initial dosing) were given 50 mg/kg and 250 mg/kg of benzene, respectively, via stomach tube, once daily, 4-5 days weekly, for 52 weeks. The animals were observed until spontaneous death 10-62 weeks later. A significant incidence of Zymbal gland carcinomas was observed together with an increased incidence of mammary carcinomas and leukemias. This study is significant because it is the first demonstration of the carcinogenicity of benzene in animals. In addition, the study reinforces the epidemiologic evidence of the leukemogenicity of benzene in humans.

(b) Hexachlorobenzene

Hexachlorobenzene has been shown to be oncogenic in two animal species. When fed diets consisting of 50, 100, and 200 ppm of hexachlorobenzene for life (from 50-70+ weeks), Syrian golden hamsters developed significant numbers of tumors at all dose levels in a variety of tissues including the liver, thyroid, and spleen (Cabral et al. 1977). In a later study, Cabral et al. (1979) demonstrated a much less pronounced oncogenicity (liver cell tumors) in outbred Swiss mice given 100 or more ppm of hexachlorobenzene in their diet for about 100 weeks.

The accidental poisoning of over 3000 people in Turkey between 1955 and 1959 has identified a number of other hexachlorobenzene-induced toxic effects in humans; they include porphyria with concomitant cutaneous, hepatic, and neurologic effects (Cabral et al. 1979). Many of these undesirable symptoms have persisted in these individuals throughout the past twenty years. Enlarged thyroids observed in the exposed population are presently being viewed with suspicion in light of Cabral's earlier finding of thyroid tumors in hamsters. It has not been determined, however, whether goiter problems are endemic to the Turkish population as a whole. No significant incidence of any oncogenic response has yet been reported in the population under study.

(3) Metabolism in Common with Known Oncogens

The metabolic pathways of benzene and its chlorinated derivatives, illustrated in Figures 1-7 in Section III.B.1.c.(1), have many similarities. One significant similarity is that these compounds are metabolized to electrophilic arene oxides that should be able to bind covalently to nucleophilic centers in vital cellular macromolecules. Experimental evidence of this capability is the covalent binding of monochlorobenzene, *o*-dichlorobenzene, and several other halogenated benzenes to cellular proteins (Reid and Krishna 1973).

Since benzene is metabolized to an arene oxide, and since benzene has been shown to bind covalently to DNA (Lutz and Schlatter 1977), its oncogenicity may be attributable to the formation of covalent bonds with cellular nucleophiles (see also discussion under part (1), Mutagenic Activity, of this subsection), and it is a reasonable assumption that chlorinated benzenes have the metabolic potential to initiate the same process.

(4) Tumor Enhancement Potential

Preliminary evidence suggests that the chlorinated benzenes and/or their metabolites may enhance the development of tumors in animals exposed to carcinogens. Several phenolic metabolites of the chlorinated benzenes have been shown to promote skin oncogenicity in mice, while the structurally related hexachlorobenzene has been implicated as an enhancing factor in the induction of hepatic cancer in mice. These studies are discussed briefly below.

Phenol, 2-chlorophenol, 3-chlorophenol, and 2,4,5-trichlorophenol were evaluated by Boutwell and Bosch (1959) as potential promoters of oncogenicity in mice. The animals were painted for 12 to 24 weeks with the promoter following topical administration of one dose of the initiator dimethylbenz(a)anthracene. They were observed for periods of up to 40 weeks. The experiment revealed promoter activity for all four compounds. This activity is relevant to the chlorinated benzenes because skin has been shown to contain the enzyme system capable of hydroxylating aromatic compounds to phenols (Pannatier et al. 1978). 2-Chlorophenol and 3-chlorophenol are known metabolites of monochlorobenzene, and 2,4,5-trichlorophenol is a metabolite of 1,2,4-trichlorobenzene.

Hexachlorobenzene apparently enhances the hepatocarcinogenicity of polychlorinated terphenyls in ICR mice (Shirai et al. 1978). While hexachlorobenzene is an oncogen in long-term tests (Cabral et al. 1977, 1979), in a short-term study (24 weeks of treatment followed by 16 weeks of observation) by Shirai et al., hexachlorobenzene at 50 ppm in the diet was not observed to give an oncogenic response. The incidence of hepatocellular carcinoma in mice fed polychlorinated terphenyls (250 ppm) plus hexachlorobenzene (50 ppm) for 24 weeks was found to be significantly higher ($p < 0.01$) than when the same dose of polychlorinated terphenyls was fed alone (30.8 percent and 14.3 percent, respectively). The authors suggest that the enzyme-inducing ability of hexachlorobenzene may be responsible for the apparently enhanced carcinogenicity of the polychlorinated terphenyls, or that if the hexachlorobenzene is weakly carcinogenic toward these mice, then the two agents may act synergistically to induce the liver tumors. However, the lack of information as to the mechanism by which hexachlorobenzene causes the observed effect makes it difficult to assess the significance of this information for the chlorinated benzenes, and it can only be considered suggestive.

2. Decision

There is considerable evidence that suggests that chlorinated benzenes may be oncogenic. This evidence includes:

a. Case reports of leukemia in humans exposed to the chlorinated benzenes;

b. Mutagenic activity;

c. Structural relationships;

d. Metabolism in common with known oncogens; and

e. Reports of tumor-enhancement potential of chlorinated benzene metabolites.

EPA believes the weight of this evidence demonstrates that exposure to chlorinated benzenes may present an unreasonable risk of oncogenic effects in humans.

The mutagenic activity of chlorinated benzenes has been discussed in Section III.F. Evidence for oncogenicity or leukemogenicity of chemicals structurally related to chlorinated benzenes includes over 100 human case reports and results of an animal study for benzene, and hamster and mouse studies for hexachlorobenzene. Benzene appears to undergo metabolic transformations similar to those found for many chlorinated benzenes, such as conversion to phenolic and catecholic products and to mercapturic acids(see Section III.B.1.c.(1).). It is likely that such products are formed by way of intermediate epoxides, which, in addition to producing the metabolites just referred to, can react with nucleophilic macromolecules such as DNA and protein. Tumor-promoting potential in mice has been observed for the chlorinated benzene metabolites 2-chlorophenol, 3-chlorophenol, and 2,4,5-trichlorophenol.

Although the above information suggests oncogenic potential for chlorinated benzenes, it is not sufficient for a determination of oncogenic hazard. Mutagenic activity as determined by short-term *in vivo* and *in vitro* testing cannot always be correlated with oncogenic activity in mammalian species. Similarly, although the likelihood that the chlorinated benzenes as a group may be oncogenic is supported by structural relationships, such evidence in the absence of test data on any chlorinated benzenes is deemed insufficient to allow for the adequate evaluation of oncogenic hazard for the chlorinated benzenes. EPA concludes on the basis of this evidence that evidence is insufficient to determine whether exposure to chlorinated benzenes presents a risk of oncogenicity.

3. Proposed Testing

EPA proposes that the chlorinated benzenes, with the exceptions noted below, be tested for oncogenicity in a two-year study in rodents according to the EPA guidelines proposed in the Federal Register, May 9, 1979 (USEPA 1979b). The guidelines call for the use of two species of rodent in the study, the rat and the mouse. Because the Maltoni and Scarnato study has recently found Sprague-Dawley rats to be sensitive to the oncogenic effects of benzene (a structurally related compound), EPA is proposing the use of this strain of rats in the proposed tests.

Monochlorobenzene, o-dichlorobenzene, and p-dichlorobenzene are not included in this proposed oncogenicity testing because they are being tested under the direction of the National Cancer Institute. While the NCI bioassay protocol differs from the oncogenicity testing standards proposed by EPA, EPA is tentatively accepting these differences in testing approaches. When the results of the NCI tests become available, the Agency will include them in its continuing evaluation of these chemicals.

H. Epidemiology

At this time, the EPA is not in a position to develop a test rule for an epidemiology study on chlorinated benzenes because the Agency lacks specific information on a suitable cohort. The EPA is proposing a rule under Section 8(a)(2)(F) of TSCA for chlorobenzenes as well as other ITC chemicals. Section 8(a) authorizes EPA to obtain readily accessible information from the files of manufacturers, processors, and importers on use, production, and worker exposure for specified chemicals. This information will play a key role in the identification of a cohort for epidemiologic studies. If a suitable cohort can be found, the Agency will decide after reviewing any available results from Section 4 testing whether to propose that an epidemiology study be undertaken.

IV. Materials to be Tested and Justification for Sampling

EPA is proposing that a representative sample of chemicals in the chlorinated benzenes group be tested initially. This sample consists of the following chemicals:

1) Monochlorobenzene
2) 1,2-Dichlorobenzene (ortho-Dichlorobenzene)
3) 1,4-Dichlorobenzene (para-Dichlorobenzene)
4) 1,2,4-Trichlorobenzene
5) 1,2,4,5-Tetrachlorobenzene
6) Pentachlorobenzene

The chlorobenzenes used in health effects testing should be substantially free of potentially interfering impurities in order that the test results can be interpreted as unambiguously as possible (for a general discussion of factors involved in the selection of test materials, see the Preamble to the proposed Chlorinated Benzenes test rule). The EPA believes that this condition can be met by testing chlorobenzenes at 99.9 percent or greater purity, with a benzene content of no greater than 0.05 percent and a hexachlorobenzene content of no greater than 0.05 percent; these limits are imposed because benzene and hexachlorobenzene have toxic effects that could lead to ambiguous test results. Commercial monochlorobenzene has been reported to contain below .05 percent benzene (Section II.A.1), and EPA believes that this level of benzene will not interfere with the proposed toxicity testing. The Agency also feels that the amounts of benzene in purified samples of the dichlorobenzenes and higher chlorobenzenes are not likely to exceed 0.05 percent. EPA further believes that a hexachlorobenzene content of 0.05 percent will not confound the test results, and that these criteria can be satisfied without excessive difficulty by the purification of commercially available materials. Thus, monochlorobenzene is or has been commercially available at a purity of 99.9 percent (see Section II.A.). Corresponding figures for other members of the sample are 99.0 percent for o-dichlorobenzene, 99.95 percent for p-dichlorobenzene, 100 percent for 1,2,4-trichlorobenzene, and 97.0 percent for 1,2,4,5-

tetrachlorobenzene; no figure was found for pentachlorobenzene. EPA welcomes comment on the ease of further purification of the less pure materials to the 99.9 percent figure.

The Agency's decision to propose testing of a representative sample rather than testing of all 11 category members rests in part on the chemical nature of the category and in part on available data on the biological effects of chlorinated benzenes.

As discussed in Section I.A.2. of this Chlorinated Benzenes Support Document, the structural relationships among the chlorinated benzenes lead to the expectation of regular progressive changes in properties going through the series from mono- to pentachlorinated benzene, with the discontinuities that arise from different isomeric arrangements of chlorine and hydrogen atoms being relatively minor in comparison with the overall trends. This expectation is supported by trends in physicochemical data of which several appear in Table 1, Section I. Thus, in proceeding from monochlorobenzene to pentachlorobenzene, densities, boiling points and partition coefficients show a gradual increase, while water solubility decreases.

Since physicochemical properties determine, in a complex fashion, the biological effects of a substance, the observed regularity in these properties of the category provides a basis for expecting that biological data on a well-chosen sample of category members can be used to characterize the biological behavior of the untested members. This is not to imply that all category members will necessarily have identical effects or similar potencies for a given effect. In choosing the category test sample, an important factor is that, with increasing chlorination, chlorobenzenes will be more resistant to metabolic attack and more likely to be retained in body tissues (see Section III.B.1.c.(1), Metabolism). Thus a sample including only mono- and dichlorobenzenes would be unrepresentative because it would include only the compounds most subject to metabolic attack and least likely to be stored in tissues. EPA is, therefore, proposing a test sample that includes all levels of chlorination. Further, the Agency believes that relative production volume should be an important factor in the sample selection. Applying these two criteria leads to the choice of monochlorobenzene, o- or p-dichlorobenzene, 1,2,4-trichlorobenzene, 1,2,3,4- or 1,2,4,5-tetrachlorobenzene, and pentachlorobenzene. EPA has decided to include both o- and p-dichlorobenzene in its test sample for two reasons. First, both have widespread general population exposure. Second, it seems prudent to include more than one isomer for at least one level of chlorination in order to provide information on to what extent the toxic effects of chlorobenzenes may be affected by the distribution of chlorine atoms. The 1,2,4,5-isomer of tetrachlorobenzene was chosen because its production is somewhat higher than that of the 1,2,3,4-isomer, and because there is not a more compelling reason to distinguish between them.

The six sample chemicals thus represent all levels of chlorination, the full range of physicochemical properties, and compounds having the highest commercial production among the chlorinated benzenes. Available data on chlorinated benzenes not included in the testing sample will serve as additional data points for evaluation of chlorobenzene toxicity when the test results become available.

V. Route of Administration

The selection of the route of administration of a test substance emphasizes the following considerations: (a) the physical and chemical properties of the test substance, such as volatility under conditions of probable or actual human exposure, (b) the predominant portal(s) of entry of the test substance in man, (c) the practicability of experimentally approximating the probable conditions of human exposure, given the physical and chemical constants of the test substance and the relative adaptability of the test species to the proposed route of administration.

For subchronic, morphological teratogenicity, reproductive effects, behavioral teratogenicity and neurotoxicity testing, EPA is proposing that monochlorobenzene be tested with inhalation as the route of administration. Monochlorobenzene is a volatile liquid, used primarily as a solvent and as an intermediate for the synthesis of chloronitrobenzenes. It appears that inhalation would be the most likely exposure route for humans. It is proposed that ortho- and para-dichlorobenzene likewise be tested for these effects with inhalation as the route of administration. Both of these compounds are used in a variety of household products. para-Dichlorobenzene is a solid that sublimes readily. Inhalation is the most likely exposure route for humans for the two dichlorobenzenes in both the occupational setting and in the home. It is proposed that 1,2,4-trichlorobenzene, a liquid, be tested with oral gavage as the route of administration for the teratogenicity and acute neurotoxicity studies. It shall be administered in the diet for the subchronic, oncogenicity, reproductive, and subchronic neurotoxicity tests. This compound is partly used as a dye carrier. Upon completion of the dyeing process, the carrier is removed from the fabric and disposed. 1,2,4-Trichlorobenzene has been identified in drinking water and it appears that the most likely exposure route for humans would be orally through the water supply. It is proposed that 1,2,4,5-tetrachlorobenzene and pentachlorobenzene, both crystalline solids, be mixed in the diet for administration to the animals for the purposes of subchronic, oncogenicity, reproductive, and subchronic neurotoxicity studies and be administered by oral gavage for acute neurotoxicity studies. In the case of 1,2,4,5-tetrachlorobenzene, the route of administration for both morphologic and behavioral teratogenicity studies should be oral gavage; for subchronic studies, it should be mixed in the diet. For pentachlorobenzene, gavage is the proposed route for behavioral teratogenicity (morphologic teratogenicity and subchronic testing are not proposed for pentachlorobenzene). In teratogenicity studies, the test chemical should not be added to the feed or water since reduction in food or water intake may seriously compromise the value of the study. 1,2,4,5-Tetrachlorobenzene and pentachlorobenzene have been found in fresh water fish and in herring gull eggs while pentachlorobenzene has been found in many foods. Therefore, the most likely exposure route for humans is orally through the food supply.

Appendix A
Use of Information From The TSCA Inventory

The TSCA Section 8(b) Chemical Inventory includes both nonconfidential and confidential information. Because TSCA Section 14 prohibits the disclosure of confidential data with certain exceptions, only non-confidential data have been used in this support document.

There are a number of qualifications associated with the use of non-confidential Inventory information. Obviously, because a manufacturer or importer could claim any or all of the information submitted as confidential*, the non-confidential data exclude any confidential information. Another qualification is that production volumes are reported for only one year, 1977. Finally, production and imports are reported in ranges rather than in specific figures, which not only increases uncertainty but makes meaningful aggregation of data difficult. (A thorough discussion of the meaning and the limitations of the chemical data is available in the Introduction in Volume 1 of the published TSCA Chemical Substance Inventory. A thorough discussion of the meaning and the limitations of the manufacturer's data is contained in the Inventory Reporting Regulations (40 CFR 710).)

Nevertheless, the publicly available Inventory data are often a useful addition to other information about a substance. In the present document, they have been used particularly to supplement fragmentary or out-of-date information from other sources. Two conventions were adopted for presenting Inventory information: 1) Where the number of importers or manufacturers of a substance is given, those who specifically reported no activity for the chemical in 1977 are not included. In a few cases, numbers of nonactive companies are also given but always as an explicit separate item; 2) Production volumes are stated in two ways. Where the highest range of production reported for a substance is 100,000 to one million pounds or lower, either a minimum figure is given or an aggregated figure is cited which is essentially a crude order-of-magnitude estimate. In other cases, the range is stated rather than an aggregate.

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*A manufacturer or importer could claim any of the following items as confidential: company name; site; specific chemical identity; whether the chemical substance is manufactured, imported or processed; whether the activity in the chemical is site-limited; and the quantity manufactured, processed, or imported.

As an example, take the statement that, according to Inventory data, six manufacturers produced substance A in 1977, with two reporting production of 10 million to 100 million pounds. This means that six manufacturers who did not claim their identities confidential reported production in 1977. There may have been others who reported manufacturing substance A since 1974 but who made none in 1977. There may also have been one or more others who reported as both producer and importer but did not specifically assign the volume reported to either activity (in the support document, such cases have sometimes been included as a separate item). Finally, although at least 20 million pounds of production of substance A has been reported, there may have been additional 1977 production by one or more of the six manufacturers that cannot be disclosed.

Appendix B
Proposed Mutagenicity Testing Sequence

In recent years, mutagenicity experts have discussed and provided guidance on hazard estimation procedures for determining if a chemical is a potential human mutagen. The following describes the basis for EPA's approach to mutagenicity testing under Section 4 of TSCA.

Four major reports on the hazards of environmental mutagens were issued between 1975 and 1979 (Drake 1975, Flamm 1977, NAS 1977, McElheny and Abrahamson 1979). In 1978 the Office of Pesticide Programs proposed Guidelines for Registering Pesticides in the U.S. (USEPA 1978b). Addendum III to these guidelines, "Criteria for Evaluating the Mutagenicity of Chemicals," contains a discussion of the scope and nature of the human genetic disease burden. The Preamble to the guidelines contains EPA's rationale for using mutagenicity data derived from non-human test systems.

These reports agree that to perform a mutagenicity hazard estimation for humans, scientists must first demonstrate that a substance and/or its metabolite(s) cause heritable gene or chromosomal mutations (the two classes of mutagenic damage which have been shown to be responsible for a portion of human genetic disease) and that the mutagenically active form can reach the genetically significant target molecules in mammalian germinal tissue.

A discussion of the principles and practices of mutagenicity testing in terms easily understood by persons unfamiliar with mutagenicity is presented in the EPA's booklet "Short-Term Tests for Carcinogens, Mutagens, and Other Genotoxic Agents" (Trontell and Connery 1979).

The following points are essential to such a rationale and are generally accepted by experts in the field of mutagenesis (see e.g., Drake 1975, Flamm 1977, McElheny and Abrahamsom 1979). They are:

(1) All organisms (except for a few viruses) have DNA as the genetic material, which contains all of the information necessary for survival and reproduction.

(2) The DNA code is the same in all organisms.

(3) The cellular machinery for decoding the information stored in the DNA code is similar among all organisms.

(4) Eukaryotic cells contain nuclei within which DNA, complexed with protein, forms complex bodies called chromosomes. Prokaryotic organisms lack nuclei, and their chromosome structure differs from that of eukaryotic organisms.

(5) Unless a mutation occurs, the information in DNA is faithfully replicated in each cell generation in both unicellular organisms and somatic and germ cells of multicellular organisms.

(6) DNA can be altered by chemicals. If this damage is repaired properly there is no mutation. If it is repaired with error or not repaired prior to replication of DNA, mutation can result. A single lesion in DNA may lead to a mutation.

(7) Point mutations usually involve changes in the bases of the DNA chain. The replacement of one purine or pyrimidine nucleotide by another is called base pair substitution; insertion or deletion of a base pair into the DNA chain is called a frameshift mutation.

(8) Breaks in DNA may lead to structural chromosomal aberrations.

(9) Disturbances in the distribution of individual chromosomes or chromosome sets can occur during cell division and result in numerical chromosomal aberrations.

(10) Mutations are generally considered to be deleterious to an organism and to result in decreased survival and reproduction. Although not all mutations are deleterious (e.g., the Ames test measures a mutation which is advantageous to the organism), it is impossible to predict the effect of an unfamiliar alteration in the genome.

Given the ubiquitous nature of DNA as the genetic material, the universality of the genetic code, and the similarity in response of genes and chromosomes of various lifeforms, a rationale for using the results from different test systems develops. Humans, as well as bacteria, fungi, and higher organisms suffer DNA damage and gene mutations; man, as well as other eukaryotes, shows structural and numerical chromosomal aberrations. For these reasons, cells of any species may be used to detect genetic changes and to predict genetic change or damage in other species, and *in vitro* testing can thus provide an initial indication of mutagenic potential.

1. Test for Gene Mutations

As just noted, a potential for causing gene mutations can be detected by subjecting a substance to *in vitro* tests.

The ability of a substance to cause heritable mutations can be detected by a sex-linked recessive lethal test in *Drosophila melanogaster*. The frequency of induced mutations is also determined in this test.

In order to show that a suggested mutagen can reach and interact with mammalian germinal tissue, the mouse sperm alkylation test can be carried out. This test gives a measure of the relationship between mammalian body exposure to a mutagen and the resulting mammalian gonadal dose. Methods for analysis of mouse sperm DNA alkylation using radiolabelled alkylating agents have been published (Sega et al. 1974). To estimate genetic risk from a dose measured as alkylations per nucleotide in the germ cells (a large fraction of known mutagens either alkylate DNA or are metabolized into alkylating agents), it is necessary to determine a dose-response relation in an experimental system that can be thoroughly analyzed for mutations induced in the different germ cell stages. Methods for determination of dose of alkylating agents measured as alkylations per nucleotide in the germ cells of Drosophila melanogaster have been developed (Lee 1978, Aaron and Lee 1978). With the combination of the mouse and Drosophila sperm alkylation systems, mammalian body exposure can be related to mutagenic potential using molecular dosimetry as a bridge between the two systems.

In summary, in vitro bacterial mutagenesis assays provide an indication of basic mutagenic potential. In the case of a test for DNA damage and repair, the test indicates whether the test substance can interact with DNA without indicating whether the interaction can lead to mutagenesis. The Drosophila sex-linked recessive lethal test detects heritable gene mutations in an insect system amenable also to molecular dosimetry. The mouse sperm alkylation test measures the ability of a given chemical and/or its metabolites to reach and to interact with the DNA of mammalian germinal tissue. This information will be used by EPA to estimate mutational risk to mammals from exposure to chlorinated benzenes and ultimately to assess the genetic hazard of human exposure.

It is possible that an agent capable of producing heritable gene mutations in mammals would not be detected in Drosophila because of species differences between insects and mammals. In such an instance, a positive result in a second system such as mammalian cell culture, together with a demonstration of the ability of the chemical to reach and interact with mammalian germinal tissue DNA, is sufficient evidence to classify a chemical as a potential human mutagen. Data gained from alkylation of the DNA in mammalian cell cultures may be used to estimate risk to man in a manner analogous to that described above for Drosophila if data cannot be collected from the insect system.

If it cannot be shown that a chemical or its reactive metabolites can reach and interact with mammalian germinal tissue DNA, there is no evidence the chemical is a potential human mutagen, and hazard assessment is unwarranted.

A testing scheme for chlorinated benzenes based on the foregoing discussion is shown in Figures 1-4. The first test in the in vitro assay (Figures 2-4) utilizes Aspergillus nidulans, since three of the chlorobenzenes have produced positive results in this system and testing of chlorobenzenes in the Salmonella/microsomal system has been negative (a detailed discussion of existing test data for chlorinated benzenes appears in Section III.F. of this Support Document). If the Aspergillus assay is negative, the higher chlorinated benzenes should be tested for activity in both mammalian cell culture and DNA repair assays before concluding that they do not exhibit mutagenic potential. Not all compounds are proposed to be tested in the entire test battery because some compounds have been adequately characterized in some tests and can be started further along in the sequence. Thus, testing of monochlorobenzene begins with the sex-linked recessive lethal test in Drosophila because this agent has already been adequately tested in assay systems for the induction of point mutations in bacteria and fungi. On the other hand, EPA does not believe that monochlorobenzene should be tested in mammalian cells in culture because this agent has been adequately tested in this system and found to be inactive.

EPA does not plan to require a mouse specific locus test for the chlorinated benzenes to demonstrate the induction of heritable gene mutations. The mouse sperm alkylation and Drosophila sperm alkylation tests were both designed to detect the ability of known alkylating agents to react with the DNA of germinal tissue and are extremely sensitive (Sega et al. 1974, Aaron and Lee 1978). For alkylating agents or for those agents

MONOCHLOROBENZENE

Gene Mutation Testing Scheme

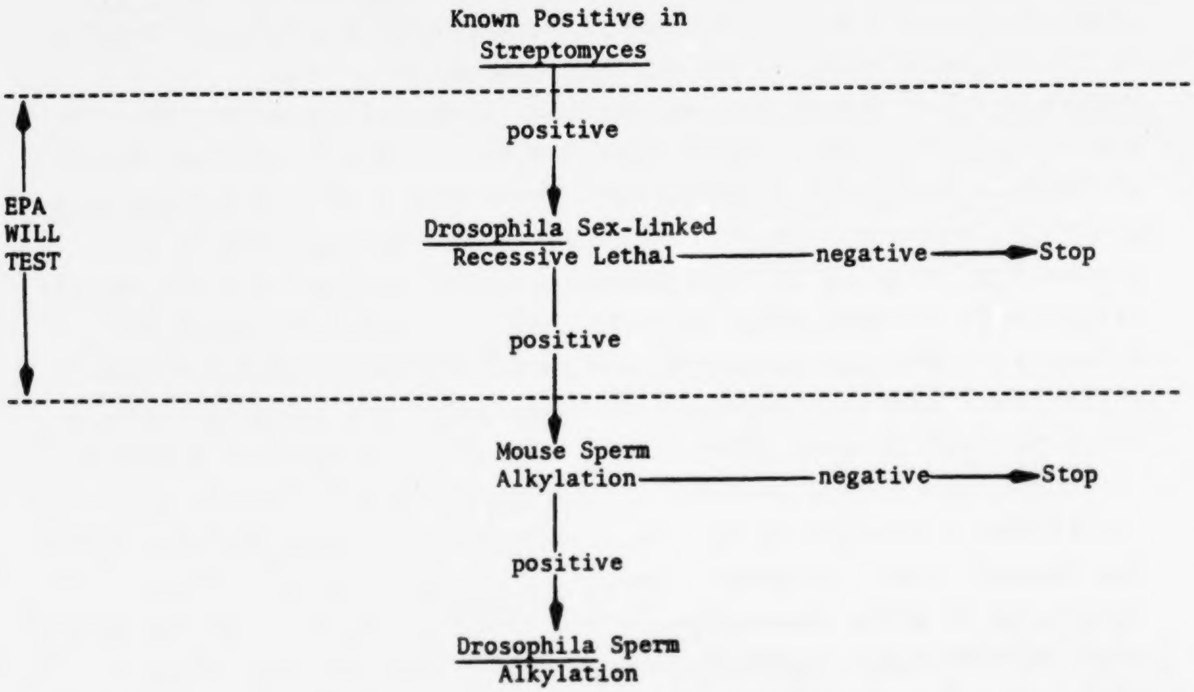
Known Positive in Streptomyces
positive
EPA WILL TEST
Drosophila Sex-Linked Recessive Lethal
negative
Stop
positive
Mouse Sperm Alkylation
negative
Stop
positive
Drosophila Sperm Alkylation

FIGURE 1

o- and p-DICHLOROBENZENE

Gene Mutation Testing Scheme

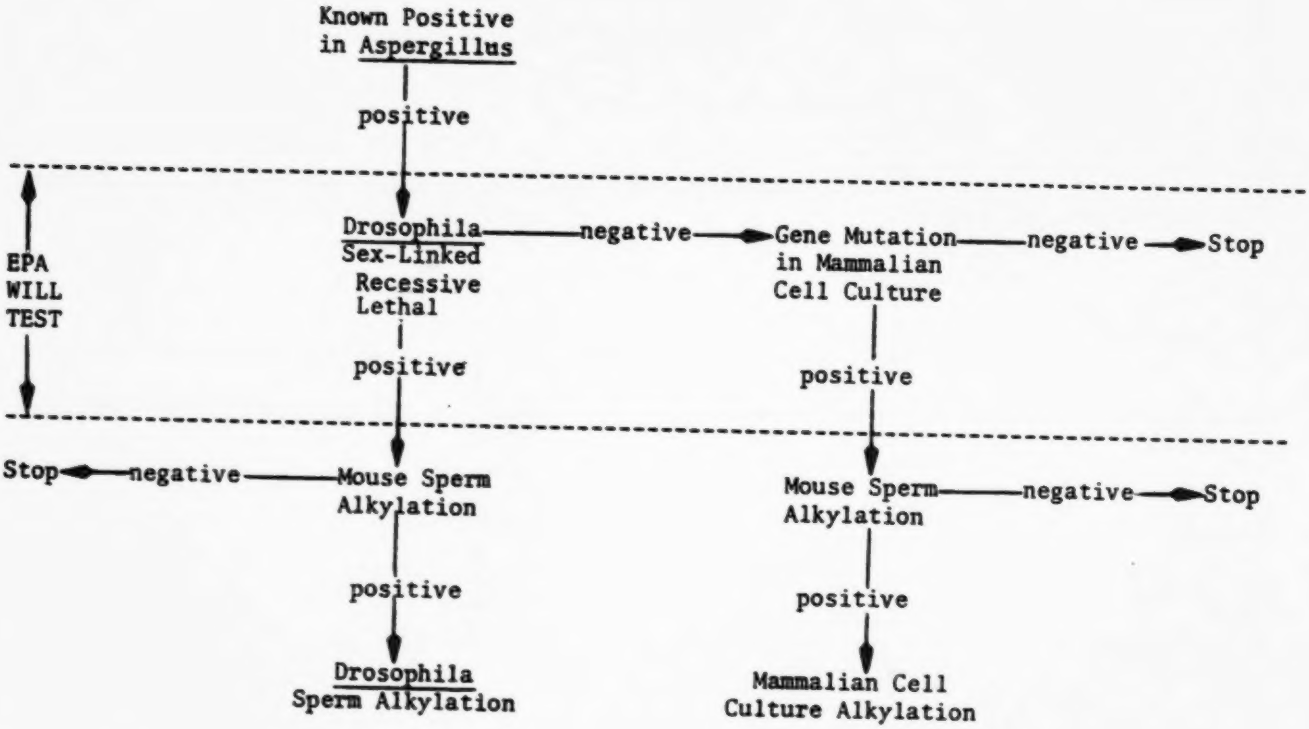

FIGURE 2

TRICHLOROBENZENE AND HIGHER

Gene Mutation Testing Scheme I

(Positive *Aspergillus* Assay)

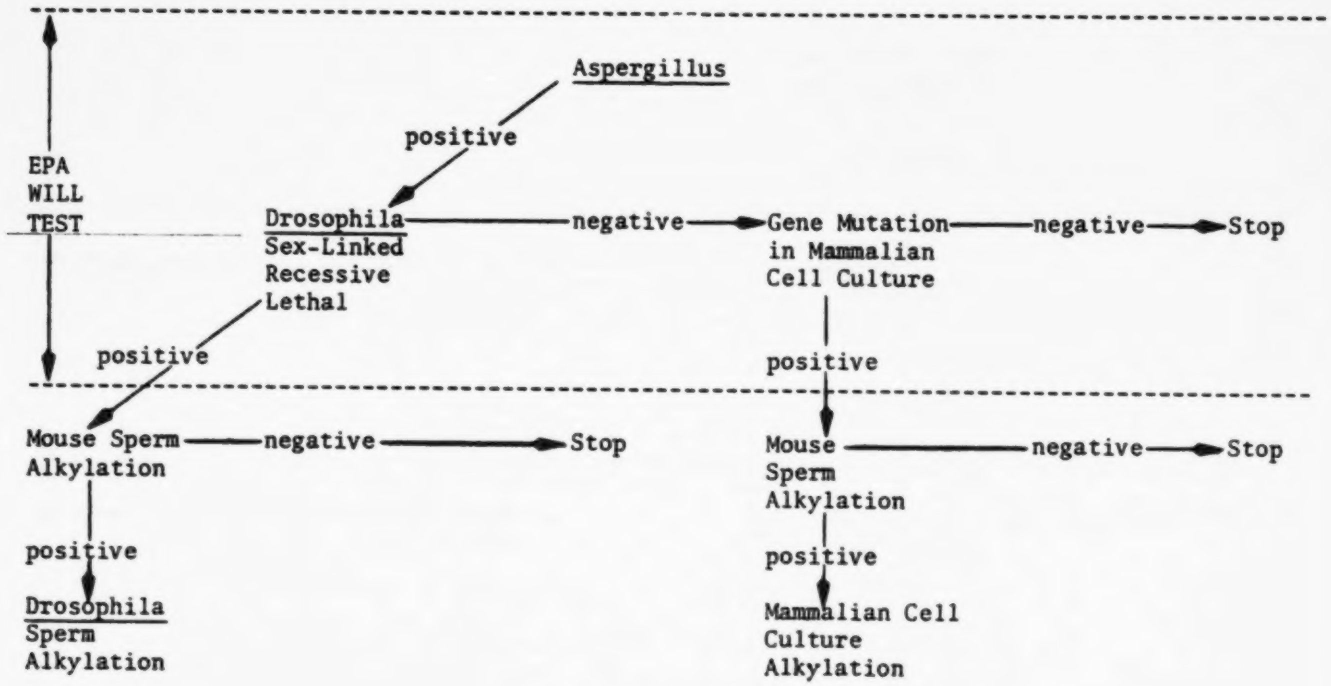

FIGURE 3

TRICHLOROBENZENE AND HIGHER

Gene Mutation Testing Scheme II

(Negative Aspergillus Assay)

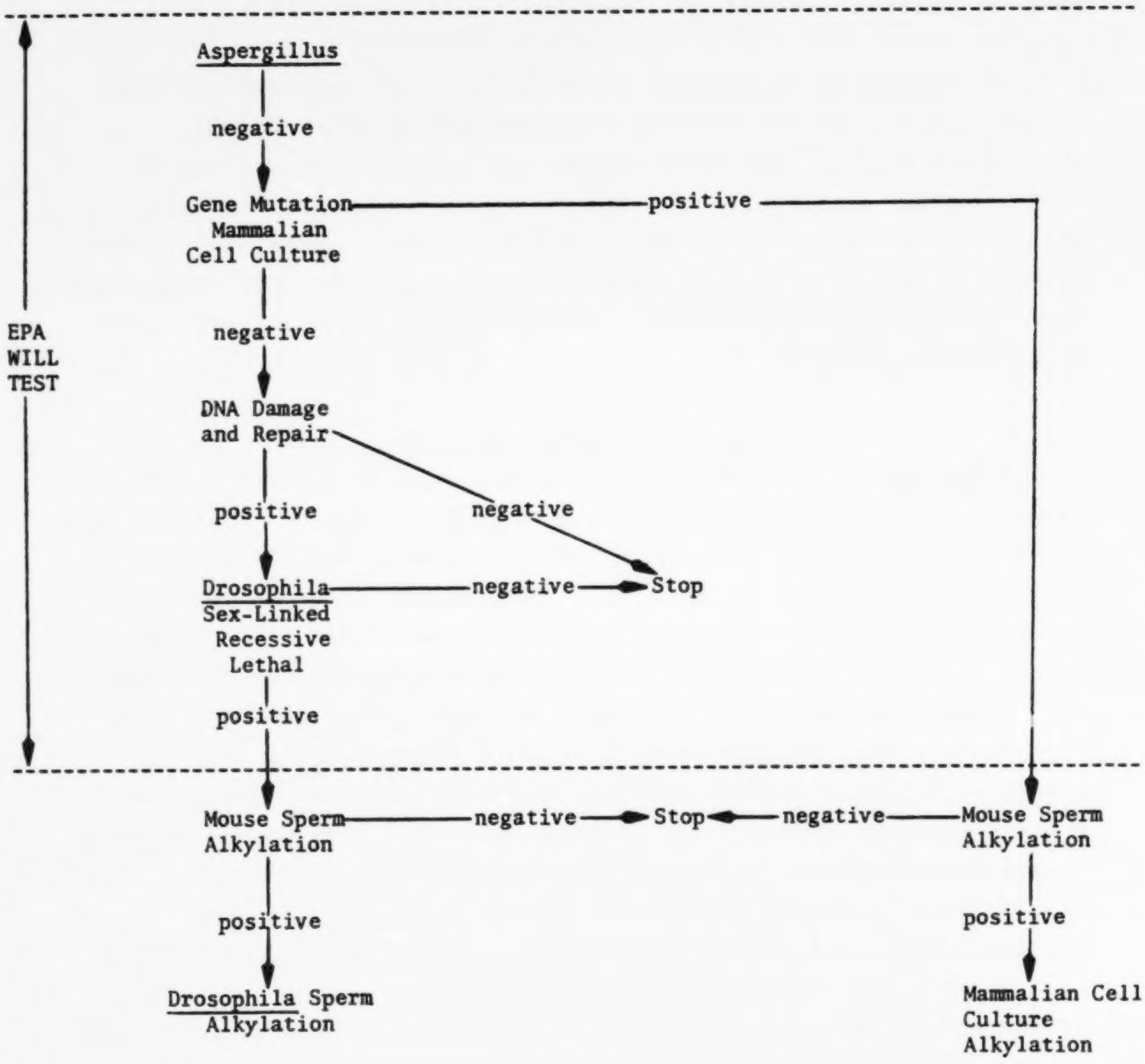

FIGURE 4

such as chlorinated benzenes that are metabolized to alkylating intermediates (see Section III.B.), DNA alkylation may possess superior sensitivity to the mouse specific locus test. This test is appropriate, therefore, for measuring the ability of the chlorinated benzenes to interact with the DNA of germinal tissue. For these reasons, EPA considers a mouse specific locus test on the chlorinated benzenes not to be warranted at this time.

As discussed in Section III.F.3, EPA will sponsor all gene mutation tests on chlorinated benzenes except the two DNA alkylation tests. EPA will follow its proposed test standards (USEPA 1979c) for all tests that it sponsors except mammalian cell culture alkylation. Protocols for rodent sperm alkylation, _Drosophila_ alkylation, and mammalian cell culture alkylation have been published by Sega et al. (1974), Aaron and Lee (1978), and Aaron et al. (1980).

2. Tests for Chromosomal Aberrations

Chromosomal aberrations may be detected in a variety of animal and plant systems employing both _in vitro_ cell culture and whole animal techniques (Flamm 1977). Because EPA is unaware of tests on chlorinated benzenes for chromosomal aberrations using mammalian systems, the Agency intends to test these substances for chromosomal aberrations in a sequence beginning with a test using mammalian cells in culture. Because it is possible that some agents that are not detected in _in vitro_ systems may be detected in whole animal systems, the sequence includes a test for chromosomal aberrations _in vivo_ following a negative _in vitro_ cytogenetics assay. No further testing for chromosomal aberrations is indicated if both the _in vitro_ and _in vivo_ cytogenetics tests are negative. A positive cytogenetics assay is followed by a dominant lethal test to demonstrate the effect of the chlorinated benzenes on germinal cell chromosomes. Brewen et al. (1975) have shown that the incidence of chromosomal breaks at first cleavage of the fertilized egg is proportional to the number of dominant lethals that occur after treatment and mating.

No further testing for chromosomal aberrations is done if the dominant lethal test is negative. A heritable translocation test shows the ability of a chemical to induce heritable chromosomal aberrations. This test, therefore, can be used not only to detect potential mutagens but also for purposes of assessing mutagenic hazard. A positive dominant lethal assay is thus followed by a heritable translocation test, the results of which will be used for hazard assessment. No further testing for chromosomal aberrations is indicated if the heritable translocation test is negative.

The test sequence for chromosomal aberrations proposed by EPA for the chlorinated benzenes is shown diagrammatically in Figure 5. As discussed in Section III.F.3, EPA will sponsor all chromosomal aberration studies on chlorinated benzenes except the heritable translocation assay. The standards EPA intends to follow for all these tests except the *in vitro* cytogenetics have been proposed by EPA (Trontell and Connery 1979). The Agency is seeking comment on the proposed sequence from all interested parties.

CHLORINATED BENZENES

Test Scheme for Chromosomal Aberrations

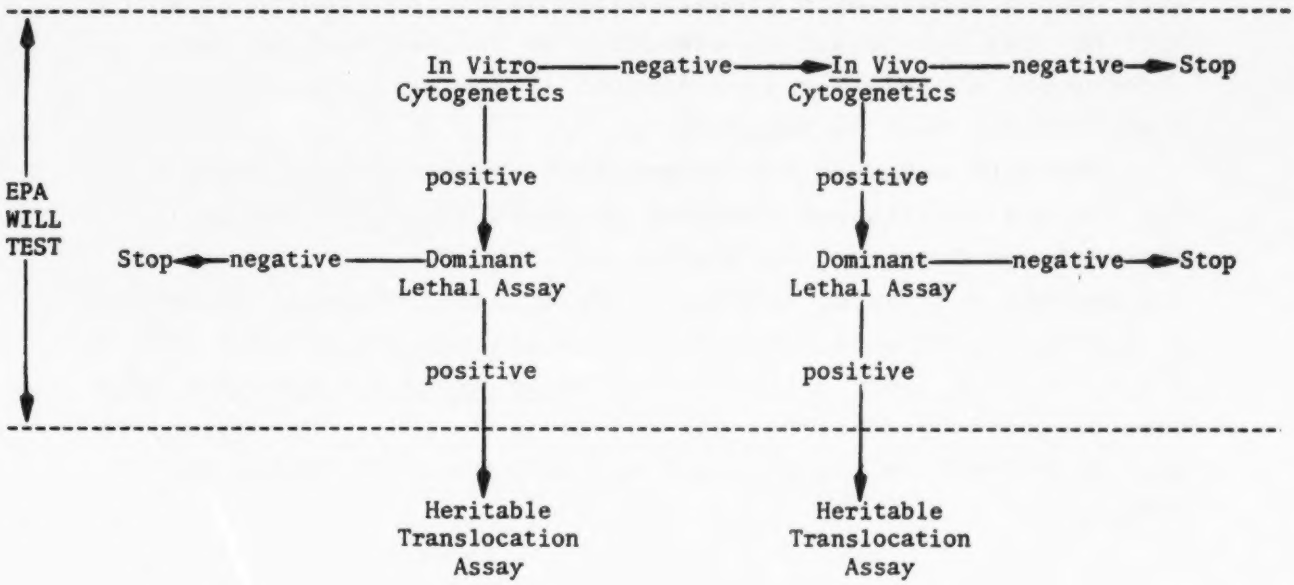

FIGURE 5

Appendix C

Occupational Exposure Limits for Chlorinated Benzenes

The American Conference of Governmental Industrial Hygienists has recommended the following threshold limit values (TLV), expressed as a time-weighted average (TWA) or short-term exposure limit (STEL) as indicated (ACGIH 1978). The TWA for the first three compounds listed have been adopted as standards by the Occupational Safety and Health Administration (USOSHA 1974).

monochlorobenzene	75 ppm	(350 mg/m^3)	TWA
o-dichlorobenzene	50 ppm*	(300 mg/m^3)	TWA
p-dichlorobenzene	75 ppm	(450 mg/m^3)	TWA
p-dichlorobenzene	110 ppm	(475 mg/m^3)	STEL
1,2,4-trichlorobenzene	5 ppm*	(40 mg/m^3)	TWA

* mixture notation--where mixtures are used, the limit recommended for the most toxic compound must be taken into consideration when evaluating the exposure.

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